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Biomolecular Identification of Ancient *Mycobacterium tuberculosis* Complex DNA in Human Remains from Britain and Continental Europe

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ABSTRACT

Tuberculosis is known to have afflicted humans throughout history and re-emerged towards the end of the 20th century, to an extent that it was declared a global emergency in 1993. The aim of this study was to apply a rigorous analytical regime to the detection of *Mycobacterium tuberculosis* complex (MTBC) DNA in 77 bone and tooth samples from 70 individuals from Britain and continental Europe, spanning the 1st–19th centuries AD. We performed the work in dedicated ancient DNA facilities designed to prevent all types of modern contamination, we checked the authenticity of all products obtained by the polymerase chain reaction, and we based our conclusions on up to four replicate experiments for each sample, some carried out in an independent laboratory. We identified 12 samples that, according to our strict criteria, gave definite evidence for the presence of MTBC DNA, and another 22 that we classified as ‘probable’ or ‘possible’. None of the definite samples came from vertebrae displaying lesions associated with TB. Instead, eight were from ribs displaying visceral new bone formation, one was a tooth from a skeleton with rib lesions, one was taken from a skeleton with endocranial lesions, one from an individual with lesions to the sacrum and sacroiliac joint and the last was from an individual with no lesions indicative of TB or possible TB. Our results add to information on the past temporal and geographical distribution of TB and affirm the suitability of ribs for studying ancient TB.

Tuberculosis (TB) has killed millions of people throughout history and is still one of the leading causes of death, with 1.4 million people having died in 2011 (WHO, 2012). TB is usually transmitted via droplet infection by coughing or sneezing, but can also be contracted through the consumption of infected meat or dairy products (Vincent and Gutierrez Perez, 1999). In humans it is most often caused by *Mycobacterium tuberculosis* but other members of the *Mycobacterium tuberculosis* complex (MTBC), namely *M. africanum*, *M. canetti*, *M. bovis*, *M. microti*, *M. pinnipedii* and *M. caprae*, have also been identified as causative agents of the disease (Gutiérrez et al., 1997; Van Soolingen et al., 1997, 1998; Aranaz et al., 2003; Kiers et al., 2008). TB has a long history and it has been suggested that this infectious disease has co-existed with humans for at least 40,000 years (Wirth et al., 2008; Comas et al. 2013). The oldest published paleopathological evidence of TB dates to 5380–4940 calBC in Germany (Nicklisch et al., 2012), and the first historical evidence to 2700 BC in China (Morse, 1967). The disease is documented throughout subsequent centuries, with a rise in prevalence during the 17th–19th centuries AD (Roberts and Buikstra, 2003:215f) explained by changes in population density as progressing urbanization, overcrowding and associated poverty facilitated spread of the pathogen throughout human communities (e.g. Armelagos et al., 2005; Lönnroth et al., 2009). Improvements in living conditions, advanced sanitation and better nutrition led to a decline of TB later in the 19th century, and the number of people suffering from the disease further dropped with the implementation of antibiotic treatment from the 1940s onwards (Roberts and Buikstra, 2003:16). Unfortunately, re-emergence of TB was observed in the 1980s and TB has since remained a major health problem in developing countries as well as in many parts of the developed world.

For many years, the study of TB and the consequent reconstruction of its origin, evolution and history was reliant solely on paleopathology, historical documents and artistic representations. In paleopathology, skeletal alterations were (and are) recognised as those described extensively in the clinical (e.g. Jaffe, 1972; Resnick, 2002a) and, subsequently, paleopathological literatures (e.g. Steinbock, 1976; Ortner, 2003). Based on 1940s and 1950s data, the skeleton is affected in about 3–5% of people with TB (Jaffe, 1972:953) but the extent of potential skeletal involvement and its manifestation in ancient populations is unknown, as our knowledge is mainly based on data from the antibiotic era, thereby obscuring individuals with minor and/or non-specific skeletal involvement, or indeed people with TB with no bone changes at all at death (Wood et al 1992; Roberts & Buikstra, 2003:125). Any bone of the skeleton can be affected, because the disease is transmitted hematogenously and via the lymphatic system from its primary focus. However, the vertebral column is the site most often affected in TB, especially the lower thoracic and upper lumbar vertebrae (Resnick, 2002a:2525ff); 25–50% of people with untreated skeletal TB will develop spinal changes. Here, lytic lesions may occur in the anterior, superior, inferior or central parts of the vertebral body which can lead to collapse of the vertebrae resulting in kyphosis, and known as Pott's disease. Destructive lesions to the joints may also suggest a tuberculous infection but these occur less frequently and usually affect only a single major joint such as the hip or knee (Resnick, 2002a:2539). It has been suggested that non-specific lesions to the skull, ribs and other parts of the skeleton may also be related to TB. For example, endocranial granular impressions and new bone formation have been associated with tuberculous meningitis (Schultz, 1999, 2001), although the classic tuberculous lesions of the skull are lytic and perforate both tables of the skull (Hackett 1976);

Lewis (2004) also indicates multiple etiologies for endocranial new bone formation. Pathological changes to the ribs in TB are usually described in the clinical literature as destructive in nature (e.g. Tatelman and Drouillard, 1953; Brown, 1980; Fitzgerald and Hutchinson, 1992). Paleopathological studies, however, have also considered new bone formation on the visceral surface of the ribs as possibly indicative of TB (Kelley and El-Najjar, 1980; Kelley and Micozzi, 1984; Roberts et al., 1994, 1998; Santos and Roberts, 2001; Matos and Santos, 2006) resulting from spread of infection from the lungs via the pleura (Jaffe 1972:990). Once again, there are many etiologies for these bone changes. Other skeletal lesions that may be related to TB include new bone formation, especially on long bones, as a result of hypertrophic pulmonary osteoarthropathy (HPOA – Resnick, 2002b:4877ff), calcified pleura (Donoghue et al. 1998) and dactylitis of the short bones of the hands and feet (Resnick 2002a:2537ff).

More than 20 years ago, invention of the polymerase chain reaction (PCR) heralded a new era in molecular genetics and provided a useful aid for identifying TB in ancient human remains. Since the first report of ancient DNA (aDNA) from MTBC (Spigelman and Lemma, 1993) ancient biomolecular data have been reported for individuals from Europe, the Middle East, North Africa and the Americas, spanning a time frame from as early as the Neolithic (e.g. HersHKovitz et al., 2008; Nicklisch et al., 2012) through the Iron Age (e.g. Mays and Taylor, 2003) and the Medieval periods (e.g. Faerman et al., 1997) up to modern times (e.g. Zink et al., 2005).

Some of these studies report MTBC aDNA amplification from unaffected bones, and teeth in skeletons showing bone lesions consistent with TB (Baron et al., 1996; Faerman et al., 1997). Others concern skeletons with non-specific lesions (e.g. Haas et al., 2000; Nicklisch et al., 2012), or none at all (e.g. Mays et al., 2002; Zink et al.,

2005). The extensive nature of this research has prompted discussion about the benefit of aDNA analyses in the study of TB (Wilbur et al., 2009; Donoghue et al., 2009). Wilbur et al. (2009) argued that destructive sampling of human remains is of little use if the intention is only to confirm a possible diagnosis of TB. Indeed, this would not prove an association of TB with lesions possibly caused by the disease (e.g. rib lesions). Further analysis shedding light on the potential infecting strain of the MTBC, however, can justify such destruction because it may add to knowledge about the evolution of TB causing agents. Wilbur et al. also addressed the need for rigorous methodology to ensure that reported detections of MTBC aDNA are authentic.

Here we describe the extent of MTBC aDNA survival in a large number of skeletons, potentially affected by TB, from different geographical regions and a range of time periods (1st–19th centuries AD). We use rigorous standards to assess the authenticity of our results, and highlight, as in past reports (e.g. Nicklisch et al., 2012), the importance of new bone formation on ribs in the study of MTBC aDNA.

MATERIALS AND METHODS

Skeletal samples

Fifty-nine skeletons from 29 British sites and eleven skeletons from eight continental European sites, spanning the 1st–19th centuries AD, were sampled during 2008 and 2010. The geographical distribution of sites is shown in Figure 1 and sample summary data are given in Table 1. More complete skeletal descriptions,

along with figures of skeletal pathological lesions, are provided as Supporting Information (Table S1 and Fig. S3). The majority of the samples for analysis were taken from ribs with new bone formation or non-pathological long bones, with only a few taken from TB affected vertebrae. Nineteen individuals were sampled from skeletal elements that did not show any pathological alteration, these samples including teeth from six individuals. Fourteen of the samples derived from skeletons that did not show pathological alterations suggestive of TB. Samples were taken with a hacksaw or electronic drill by personnel wearing protective clothing including forensic suits, hair nets, face masks and two pairs of sterile gloves. Samples were then placed in sterile plastic bags and stored under dry conditions.

Ancient DNA authentication regime

Ancient DNA analyses were performed in the aDNA laboratories of the University of Manchester and Complutense University of Madrid, Spain. Work in Manchester was carried in a suite of independent, physically isolated laboratories, each with an ultrafiltered air supply maintaining positive displacement pressure and a managed access system. All surfaces within the laboratories were periodically sterilized by UV irradiation and cleaned with 5% bleach and 70% ethanol, and all utensils and equipment were treated with DNA-Away (Molecular Bioproducts) before and after use. Items such as test tubes were UV irradiated (254 nm, $120,000 \mu\text{J cm}^{-2}$ for 2×5 min, with 180° rotation between the two exposures) before use. Aqueous solutions were similarly irradiated for 15 min. Personnel wore protective clothing including forensic suits, face masks, hair nets, goggles and two pairs of sterile gloves at all times. DNA extractions were carried out in a Class II biological safety cabinet in one laboratory within the facility, and PCRs were set up in a laminar flow cabinet in a

second, physically-isolated laboratory. Work in Madrid was carried out in physically separated laboratories for DNA extraction and PCR set-up, both UV irradiated before and after each work period. Surfaces and laboratory equipment were regularly cleaned with bleach. Personnel wore disposable laboratory coveralls, masks, caps, glasses, shoe covers and gloves. All reagents and consumables were DNase and RNase free. All procedures were carried out in a laminar flow cabinet previously cleaned with bleach and UV irradiated. Modern MTBC DNA was not present in the building in which the Manchester laboratories are located prior to completion of the aDNA extractions, and was never present at all in the Madrid laboratories. All DNA extractions were accompanied by two blanks (normal extraction but without skeletal material) per five samples (Manchester) or one blank per seven samples (Madrid). Every set of 5–7 PCRs was accompanied by at least two blanks (set up with water rather than DNA extract).

DNA analysis

To remove external contamination from bone samples, approximately 1–2 mm of the outer surface was removed mechanically, and the remaining sample UV irradiated (254 nm, 120,000 $\mu\text{J cm}^{-2}$) for 2×5 min, with 180° rotation between the two exposures, prior to crushing into a powder. Each tooth was cleaned externally by placing it, with the roots pointing upwards, in a small beaker containing sufficient 5% bleach solution to reach a level just below the root holes. After 5 min the tooth was removed, dried with a paper towel, placed in a second beaker and rinsed in Millipore water, again without inundating the root holes. After drying, a 37% phosphoric acid etching solution was applied to the tooth surface, left for 1 min, then wiped off. The

tooth was rinsed in Millipore water, dried for 10 min, and 50–100 mg dentine powder collected using a dental pick.

At least two DNA extractions were performed with each sample. In the first extraction, 250 mg of bone or 50–100 mg of tooth powder were processed using the method of Bouwman and Brown (2005) (described below as ‘protocol I’). Subsequent extractions used the method based on Rohland and Hofreiter (2007) and Rohland et al. (2010), described by Bouwman et al. (2012) (‘protocol II’). Quantitative PCR (qPCR) directed at a 63 bp product of the IS1081 insertion sequence, thought to be specific to the MTBC (Collins and Stephens, 1991; van Soolingen et al., 1992), used a forward primer 5′–TCATCGCGTGATCCTTCGA–3′, reverse primer 5′–GAGGTCATTGCGTCATTTCTT–3′ and probe 6FAM–ACCAGCAAAAGTCAATC–MGBNFQ, where 6FAM is 6-carboxyfluorescein reporter dye and MGBNFQ is molecular-groove binding non-fluorescence quencher (Applied Biosystems). A PCR mix with a total volume of 30 µl comprised 5 µl DNA extract, 1 × TaqMan Universal PCR Master Mix (Applied Biosystems), 900 nM forward primer, 300 nM reverse primer, 250 nM probe, and 1% bovine serum albumin (BSA). Positive controls to monitor amplification efficiency were run in duplicate using 1.7×10^4 to 1.7×10^{-1} genome copies μl^{-1} *M. tuberculosis* H37Rv DNA (Advanced Biotechnologies). Amplifications consisted of: UNG AmpErase incubation for 2 min at 50°C; 10 min at 95°C; 55 cycles of 15 sec at 95°C, 1 min at 60°C. Standard PCRs (Eisenach et al., 1990) directed at a 123 bp fragment of insertion sequence IS6110, also thought to be specific to the members of the MTBC (Thierry et al., 1990), were carried out in 30 µl reactions comprising 2.5–5.0 µl DNA extract, 1 × AmpliTaq Gold PCR Master Mix (Applied Biosystems), 400 nM each primer and 1% BSA. Cycling conditions were: 95°C for 7 min; 45 cycles of 1 min at 68°C, 1 min at 94°C, 10 min at 72°C. Nested

PCR was performed with those samples that did not yield the 123 bp product. This involved repetition of the first-round PCR but with 35 cycles, followed by a second-round PCR set up as described above but with the primers described by Taylor et al. (1996) and using 1 µl of first-round product. Cycle conditions were altered to 25 cycles at an annealing temperature of 58°C. PCR products of the correct size were cloned (CloneJet™ PCR cloning kit, Fermentas) into *Escherichia coli* XL1-Blue competent cells (Agilent), and inserts amplified by colony PCR in 20 µl comprising 1 × *Taq* buffer (New England Biolabs), 200 nM each primer, 200 µM dNTPs, and 0.625 units *Taq* DNA polymerase (New England Biolabs), with cycling at: 95°C for 3 min; 30 cycles of 30 sec at 94°C, 30 sec at 60°C, 1 min at 72°C; 10 min at 72°C. Colony PCR products were sequenced (GATC Biotech, Cologne). Sequences were aligned with the *M. tuberculosis* H37Rv reference sequence for IS6110 using Geneious version 6.0.3 (created by Biomatters, available from <http://www.geneious.com/>).

The initial DNA analysis of each sample was performed in Manchester. Replications of positive MTBC detections were carried out from the extraction stage in Madrid and/or from a freshly prepared extract in Manchester. Samples for which the results could not be replicated in this way were rechecked using the initial extracts.

RESULTS

A summary of the results is given in Table 2. For each sample, IS1081 qPCRs and IS6110 PCRs were carried out with two independent extracts prepared by protocols I and II in Manchester. In most cases, those samples that gave a positive IS6110 result were retested with a third IS6110 PCR using a fresh extract prepared

by protocol II in Madrid. For some samples, the third replication of an IS6110 result was performed in Manchester (indicated by footnotes 3 and 4 in Table 2).

An IS1081 qPCR was considered to give a positive result if the fluorescent signal crossed the cycle threshold no later than the 40th cycle ($Ct \leq 40$), this cut-off intended to avoid false-positives arising from non-specific amplification. According to this criterion, 15 samples gave positive results with both extracts, and a further 15 gave a positive result with one extract. Positive IS6110 detections were based on amplification of a first- or second-round nested PCR product of the correct size and whose clone sequences were identical to the IS6110 reference sequence, except possibly for a small number of differences that can be attributed to miscoding lesions in the aDNA templates (see Supporting Information Figures S1 and S2). Some samples gave agarose gel electrophoresis bands of about the correct size for the 123 bp IS6110 product but the sequences obtained from clones of these '123 bp' bands showed up to 16 differences to the IS6110 reference sequence. These samples also did not give nested 92 bp products, and were therefore not considered to be positive detections of MTBC aDNA. None of the extraction or PCR blanks gave amplification products. The presence of inhibitors in the extracts was evaluated by PCR of other targets, not described here. We found five samples (Addenbrookes 3667, Heslington East 229, Horncastle 167, 186 and 274) that contained inhibitors and which required 10^{-1} or 10^{-2} dilution before PCRs were successful. None of these samples gave specific amplification products with IS6110 PCRs. One sample (Kingsholm 96) only gave the 92 bp IS6110 product after 0.5 M betaine had been added as a PCR enhancer instead of BSA. The use of betaine was also tested with other samples but did not improve any of the results.

We identified samples as containing MTBC aDNA if clone sequences from at least two extracts matched the 123bp and/or 92bp reference sequence for IS6110. Twelve samples, corresponding to 15.6% of those tested, fell into this *definitely* positive category. We concluded that a sample *probably* contained MTBC aDNA if a 123 bp or 92 bp sequence, matching the IS6110 reference, was obtained from one of the extracts. We identified samples as *possibly* containing MTBC aDNA if at least one of the extracts provided a signal for the IS1081 qPCR only. Finally, samples were identified as not containing MTBC aDNA if neither a signal for IS1081 nor a sequence matching the IS6110 reference was obtained from any of the extracts.

The association between pathological lesions and MTBC aDNA detection is shown in Table 3. Of the 12 samples identified as definitely containing MTBC aDNA, nine came from skeletal elements displaying lesions that are considered non-specific for TB and, of those nine, five were from skeletons showing rib lesions only (Auldham 43, Ashchurch 705, Saint Amé 20, St Peter's Church 1390, St Peter's Collegiate Church 28). A further three individuals (St Peter's Collegiate Church 62, St George's Crypt 4006, Whitefriars 10466) displayed more widespread new bone formation throughout the skeleton and one individual showed destructive lesions to the sacrum and sacroiliac joint (Göttingen 13.k.36.1). Two of the 19 samples taken from unaffected parts of the skeleton gave definite evidence for MTBC aDNA (Shchekavitsa 8, Whitefriars 657). One of these was a femur sample from an individual displaying endocranial lesions (Shchekavitsa 8). The second, Whitefriars 657, was an individual with rib lesions as the only possible indication of TB; the rib sample from this skeleton gave only possible evidence for MTBC aDNA, but the mandibular molar gave a definite positive result. Ancient DNA detections in the 'probable' category were obtained for teeth from two individuals (Ashchurch 705, St

George's Crypt 4006) whose sampled ribs gave definite evidence of MTBC aDNA. One of the 14 samples from individuals without any lesions suggestive of TB gave definite evidence for MTBC DNA (St George's Crypt 5003).

DISCUSSION

A rigorous regime for MTBC aDNA detection

There have been a number of previous reports of MTBC aDNA detection from archaeological human samples of various types and ages (e.g. Spigelman and Lemma, 1993; Salo et al., 1994; Baron et al., 1996; Taylor et al., 1996, 1999; Faerman et al., 1997; Nerlich et al., 1997; Donoghue et al., 1998, 2005; Haas et al., 2000; Mays et al., 2001, 2002; Zink et al., 2001, 2003, 2005; Mays & Taylor, 2003; Hershkovitz et al., 2008; Nicklisch et al., 2012). However, Wilbur et al. (2009) have argued that the procedures used to authenticate individual detections have not been uniformly robust and that some of these reports might be insecure. To avoid this criticism, we adopted a rigorous regime for verification of PCR outcomes, aimed at providing a higher degree of confidence in the results of our survey. This regime was based on four key principles. First, we rejected the suggestion (Donoghue et al., 2009) that studies of MTBC aDNA can be carried out “without the need for a dedicated facility, with containment and filtered air” (ibid p.2802). Relaxation of this standard requirement for authentication of aDNA results (Cooper & Poinar, 2000) might be justified if the only source of modern contamination is DNA from people handling the samples, who are unlikely to carry MTBC DNA. We believe that amplicon cross-contamination is a significant problem that can lead to false-positive detections of MTBC aDNA, and therefore carried out our project in dedicated aDNA facilities using procedures designed for handling of human aDNA, and hence

intended to prevent not just contamination from personnel but also contamination by amplicon crossover. Second, we did not score positive PCRs solely from the generation of amplicons of the expected size. Specificity of IS1081 detections was ensured by using a probe-based qPCR approach, and all putative IS6110 amplicons were checked for authenticity by sequencing of cloned products. The importance of sequencing for IS6110 validation was underlined by our discovery of several amplicons of apparently correct size that did not match the IS6110 reference sequence. Third, we carried out replicates of all PCRs using fresh extracts, with a sub-set of samples replicated in an independent laboratory. Finally, we did not simply assign samples as positive or negative for MTBC aDNA presence, but instead adopted an evidence-based system in which samples could be 'probable' or 'possible' based on the results of individual PCRs.

Using this approach, of the 77 samples that we tested, we identified 12 (15.6%) as definitely containing MTBC aDNA, another 22 (28.6%) as probable and possible, and 43 (55.8%) as negative. Despite our precautions, we accept that these results are probably not entirely accurate. Although none of the extract and PCR blanks that we performed alongside genuine extractions and PCRs gave products, we cannot discount the possibility that some of our results are false positives due to contamination with modern MTBC aDNA from whatever source. This consideration applies in particular to those samples that we identify as 'probable' and 'possible', because with these samples the positive PCRs could not be replicated with independent extracts. Detections based solely on IS1081 are particularly insecure, in our view, because IS1081 is similar to other insertion sequences found in environmental mycobacteria (Picardeau et al., 1996) and hence might be present in bones as a contaminating rather than endogenous sequence. We therefore classify

those 10 samples for which we obtained a signal for IS1081 but no products for IS6110 as 'possible' and urge caution looking on these as authentic MTBC aDNA detections until further corroborating evidence can be obtained.

Conversely, it is possible that some of the samples that we place outside of the 'definite' category do in fact contain MTBC aDNA. IS6110 has been the primary target in biomolecular studies of ancient TB since the first publication in this field. Nevertheless, it is known that negative results for this target do not exclude the presence of MTBC aDNA as low and zero copy-number strains exist (Thierry et al., 1990; Yuen et al., 1993; Lok et al., 2002). Technical problems can also preclude detection of aDNA that is present in a sample. We tested our extracts for the presence of PCR inhibitors and believe that this was not a cause of false negatives in our screening, and we also examined the possibility that PCR enhancers such as betaine might improve aDNA detection. These precautions do not preclude the possibility that our results include false negatives.

Factors influencing MTBC aDNA preservation

The disparities between MTBC aDNA preservation in the different samples can be explained by a number of factors. Many of these are well documented, including burial environment (Burger et al., 1999), degree of morphological preservation (Colson et al., 1997; Götherström et al., 2002; Haynes et al., 2002), storage conditions after excavation and conservation treatments (Nicholson et al., 2002; Pruvost et al., 2007), and unequal distribution of DNA within a skeleton. One important diagenetic factor in MTBC detection concerns the vertebrae, which are of particular interest as they may display typical tuberculous alterations and hence be attractive targets for aDNA detection. In fact, none of the vertebrae that we sampled

were definitely positive for MTBC aDNA. The vertebral body surfaces can be very porous, exposing the cancellous bone of the body to the surrounding environment. Although this may not always be clearly visible macromorphologically, any water or soil infiltration of the vertebral body imposes a higher risk of DNA degradation. Infiltration may also introduce PCR inhibitors into the bone; in this regard, we note that four of the five bone samples (although a small number) which we found to contain inhibitors were vertebrae.

As well as general diagenetic factors, the etiology and pathogenesis of TB during life can have had an impact on the subsequent preservation of MTBC aDNA in a bone or dental sample from an archaeological skeleton. In order to develop skeletal lesions, whether specific or non-specific, TB has to be long-standing. Previous studies have shown that MTBC aDNA can be obtained not only from bones displaying typical TB associated lesions (e.g. destructive lesions in the spine), but also from bones with non-specific lesions (e.g. Haas et al., 2000; Mays et al., 2002; Mays and Taylor, 2002; Nicklisch et al., 2012). The latter was the case with nine of the 12 samples that we identified as definitely containing MTBC aDNA. Three of these (St Peter's Collegiate Church 62, St George's Crypt 4006, Whitefriars 10466) came from individuals displaying new bone formation on the visceral surface of the ribs along with widespread new bone formation throughout the skeleton, possibly reflecting HPOA. Another four (Auldham 43, Ashchurch 705, Saint Amé 20, St Peter's Collegiate Church 28) were from skeletons that displayed new bone formation only on the ribs, although with one of these (Ashchurch 705), vertebral involvement cannot be excluded as the vertebral column was missing (Holst, 2004). One individual (St Peter's Church 1390) displayed both new bone formation and lytic lesions on the ribs, the latter more typical for TB involvement of ribs, and one was

from an individual with destructive lesions to the sacrum and right sacro-iliac joint as well as new bone formation on the right ilium (Göttingen 13.k.36.1). Rib lesions, especially in case of periosteal new bone formation on the visceral surface, may not necessarily be caused by TB as they can result from inflammation of the lungs from other causes (Roberts et al., 1994). The endocranial lesions seen with Shchekavitsa 8, an individual that was sampled from a skeletal element not displaying lesions, may have been caused by a number of diseases leading to the new bone formation, tuberculous meningitis being one, resulting from spread of inflammation from the meninges (Schultz, 1999, but see Lewis 2004). However, whether a person in the past with meningitis without access to antibiotics could survive long enough for the bone changes to occur is still debated. Any of the individuals mentioned so far may have undergone widespread bacteremia at the time of death, a possibility also suggested for Whitefriars 657, whose tooth gave a definite MTBC aDNA detection but whose rib was only 'possible', and for St George's Crypt 5003, an individual without any signs of TB. Our results therefore suggest that vertebrae are not necessarily the best choice for biomolecular studies of ancient TB, and also indicate that MTBC aDNA may be more widespread in the skeleton than is suggested by the macroscopic evidence of the disease, as has been shown by the autopsy of extant TB victims (Zink et al., 2005).

Occurrence of TB in the past

Our results add to current knowledge of the past temporal and geographical occurrence of TB, mostly from macroscopic analyses of human remains from archaeological sites but also from historical and iconographic as well as biomolecular evidence. Most of our samples came from Britain, spanning the Roman era up to the

19th century AD. The first probable evidence of TB in Britain, a male individual displaying destructive spinal lesions, dates to 400–230 BC and was reported to contain MTBC aDNA (Mays and Taylor, 2003; Taylor et al., 2005). Further evidence of TB has been reported for the Roman period, but at a lower frequency than is indicated by the skeletal record for later periods (Roberts and Buikstra, 2003:132ff), when TB becomes one of the most prevalent diseases, reaching a peak in the 18th–19th centuries AD. Our findings for Ashchurch 705 confirm the presence of TB in Britain in Roman times and support the notion that the actual prevalence of the disease might be underestimated (Roberts and Buikstra, 2003), as we obtained probable results for three individuals not showing any pathological alterations due to TB. We obtained more definite biomolecular evidence for TB in Britain from two Medieval skeletons (Auldham 43, St Peter's Church 1390), from a time when TB was not confined to the southern and eastern parts of the country, as seems to have been the case for the Roman period (Roberts and Buikstra, 2003:132). By the 12th–13th centuries AD the disease had even reached the southeast part of Scotland, as indicated by the individual from Auldham (43). The majority of definite MTBC containing samples derived from individuals dated to the 18th–19th centuries AD, a time at which TB was one of the leading causes of death in Britain (ibid: 216).

TB has also previously been reported for other geographical regions from which we obtained samples, although for these countries there have been fewer bioarchaeological investigations and the earliest dates for the disease are uncertain. The paleopathological evidence for TB in France places the first appearance of the disease during the 4th century AD (Blondiaux et al., 1999) and further evidence, although limited in comparison to Britain, has been identified for subsequent time periods up to the 18th century AD (summarized in Roberts and Buikstra, 2003:167ff).

Biomolecular findings have been reported, for a 12th–13th century AD individual from southeast France (Dutour et al., 1999). Our definite detection of MTBC aDNA with individual Saint Amé 20 adds further evidence for presence of the disease during the 16th–18th centuries AD in northeast France. In Germany, TB was apparently present in the Neolithic with MTBC aDNA detected in individuals with Pott's disease and/or rib lesions (Nicklisch et al., 2012). Later skeletal and biomolecular evidence has been reported for a German site dated to the 14th–18th centuries AD (Zink et al., 2005) and from mummified remains from 1550–1750 AD (Lösch et al., 2008). Positive MTBC aDNA results have also been reported for autopsy material from three 19th to early-20th century AD individuals with known bone TB (Baron et al., 1996) from the historical pathological collection from which we obtained our definite positive sample (Göttingen 13.k.36.1). Finally, our findings for Shchekavitsa 8 provide evidence for the presence of TB in the Ukraine in the late-10th–12th centuries AD. We are not aware of any reports of historic TB in the Ukraine but skeletal evidence of the disease exists for Poland in the 10th–13th centuries AD (summarized in Gladykowska-Rzeczycka, 1999), and possible evidence of TB was described in an individual from a 10th–12th century AD site in southwest Russia (Rokhlin, 1965).

Conclusions

Using a rigorous technical regime designed to avoid some of the criticisms that have been made of past biomolecular studies of archaeological TB, we identified 12 samples from ancient human remains that definitely and a further 22 that probably or possibly contain MTBC aDNA. These 34 samples included only four of the 11 that had been taken from vertebrae showing lesions associated with TB, suggesting that such elements might not be good sources of MTBC aDNA. Eleven of the 12 definite

samples derived from individuals displaying lesions non-specific for TB, in particular new bone formation on the visceral surface of the ribs, and one was taken from an individual without any lesions suggestive of the disease. The majority of these samples derived from British skeletal material spanning the 2nd and 3rd up to the 19th centuries AD, but three samples derived from Germany, France and the Ukraine. Our results positively add to the discussion about whether visceral surface new bone formation on the ribs may be an indicator of TB and further support the use of ribs for the molecular study of ancient TB. Nevertheless, we emphasize that evidence of MTBC aDNA does not confirm that the lesions displayed by an individual are actually caused by TB, and similarly that failure to produce MTBC aDNA data does not exclude individuals having suffered from TB. The inconsistencies in MTBC DNA amplification success with regard to the probable and possible samples are mostly likely due to limited aDNA survival, resulting from the effect of diagenetic processes and/or the etiology and pathogenesis of TB in life. The generally low number of positive individuals revealed by our rigorous approach, especially for earlier time periods, is contrary to the much higher success rates reported in many previous studies of ancient TB.

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Figure legend

Fig. 1. Location of sites and collections for the samples used in this study. The large map displays British sites/collections, the small those within continental Europe. Map created using ArcMap™ 9.2 (ESRI).

Supporting information for Muller et al.: Biomolecular Identification of Ancient *Mycobacterium tuberculosis* Complex DNA in Human Remains from Britain and Continental Europe

Figure S1. Alignment of clones for the 123 bp (longer sequence) and 92 bp product (shorter sequence) of IS6110 from samples identified to definitely contain MTBC aDNA. Primers are removed. The reference sequence for IS6110 is given at the top. Differences to the reference sequence, most likely representing damage-derived miscoding lesions or PCR errors (Brotherton et al., 2007; Gilbert et al., 2007), are highlighted in colour. Numbers 1–4 following the sample ID denote the respective extraction (1, 1st Manchester extraction protocol I; 2, 2nd Manchester extraction protocol II; 3, Madrid extraction protocol II; 4, additional Manchester extraction protocol II). Letters a–f indicate the respective clone obtained for each amplification.

Literature Cited

Gilbert MTP, Binladen J, Miller W, Wiuf C, Willerslev E, Poinar H, Carlson JE, Leebens-Mack JH, Schuster SC. 2007. Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. *Nucleic Acids Res* 35(1):1□–10.

Figure S2. Alignment of clones for the 123 bp (longer sequence) and 92 bp product (shorter sequence) of IS6110 from samples identified to probably contain MTBC aDNA. Primer sites are removed. The reference sequence for IS6110 is given at the top. Differences to the reference sequence, most likely representing damage-derived miscoding lesions or

sample Manchester extraction protocol I, (2nd Manchester extraction protocol II). Letters a–f indicate the respective clone obtained for each amplification and letters aa–ee denote the respective clone obtained for the second amplification from the same extract.

IS6110-reference

Ashchurch 705T-2a

Ashchurch 705T-2b

Ashchurch 705T-2c

Ashchurch 705T-2d

Ashton 118-2a

Ashton 118-2b

Ashton 118-2c

Ashton 118-2d

Ashton 118-2e

Ashton 118-2f

Ashton 118-2aa

Ashton 216-2a

Ashton 216-2b

Ashton 216-2c

Ashton 216-2d

Ashton 216-2d

Horncastle 20-2a

Horncastle 20-2b

Horncastle 20-2c

Horncastle 434-2a

Horncastle 434-2b

Horncastle 434-2c

Horncastle 434-2d

Kingsholm 96-1a

Kingsholm 96-1b

Kingsholm 96-1c

Kingsholm 96-1d

Kingsholm 96-1e

Kingsholm 96-1f

Kingsholm 236-2a

Kingsholm 236-2b

Kingsholm 236-2c

Obeliai 143A-2a

Obeliai 143A-2b

Obeliai 143A-2c

Obeliai 143A-2d

Obeliai 143A-2e

Obeliai 143A-2f

St George's Crypt 4006T-2a

St George's Crypt 4006T-2b

St George's Crypt 4006T-2c

St George's Crypt 4006T-2d

Water Lane 1-2a

Water Lane 1-2b

Water Lane 1-2c

Water Lane 1-2d

Water Lane 1-2e

Water Lane 1-2f

Water Lane 1-2aa

Water Lane 1-2bb

Water Lane 1-2cc

Water Lane 1-2dd

West Thurrock 287-2a

West Thurrock 287-2b

West Thurrock 287-2c

Wheatpieces 4-2a

Wheatpieces 4-2b

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Brotherton P, Endicott P, Sanchez JJ, Beaumont M, Barnett R, Austin J, Cooper A. 2007. Novel high-resolution characterization of ancient DNA reveals C > U-type base modification events as the sole cause of post mortem miscoding lesions. *Nucleic Acids Res* 35(17):5717–5728.

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Biomolecular Identification of Ancient *Mycobacterium tuberculosis*
Complex DNA in Human Remains from Britain and Continental Europe

Romy Müller, Charlotte A. Roberts and Terence A. Brown

SUPPORTING INFORMATION

TABLE S1. Full description of skeletons sampled. (this file)

Fig. S1. Alignment of clones for the 123 bp and 92 bp products of IS6110 from samples identified to definitely contain MTBC aDNA. (Müller Fig. S1.pdf)

Fig. S2. Alignment of clones for the 123 bp and 92 bp products of IS6110 from samples identified to probably contain MTBC aDNA. (Müller Fig. S2.pdf)

Fig. S3. Images of pathological lesions displayed by the individuals investigated. (Müller Fig. S3.pdf)

TABLE S1. Full description of skeletons sampled.

Site ID	Period	Skeleton ID	Sample date	Parts of skeleton affected or possibly affected by TB	Reference
BRITISH SITES					
Kempston, Bedfordshire	Roman	3902	3 rd –4 th AD	None	Boylston and Roberts, 1996;
		3908	3 rd –4 th AD	Visceral surface woven new bone formation on ribs	Boylston et al., 2000
		3953	3 rd –4 th AD	Visceral surface woven new bone formation on ribs	
		3956	3 rd –4 th AD	Visceral surface woven new bone formation on ribs	
Addenbrookes, Cambridgeshire	Roman	3667	mid/late 1 st AD	Destructive lesions in T12 and three lumbar vertebrae (L1 and either L3 and L4, or L4 and L5), with collapse of two of the latter	Dodwell, 2008
Duxford, Hinxton Road, Cambridgeshire	Roman	24	50 calBC–140 calAD	Destructive lesions in lumbar vertebrae	Lyons, 2011
Poundbury, Dorset	Roman	131		Lesions consistent with Pott's disease affecting three vertebral bodies with collapsing and anterior bony ankylosis	Farwell and Molleson 1993; Lewis 2011
		228		Lytic lesions and visceral surface woven new bone formation on ribs	
		257		Visceral surface woven new bone formation on ribs	
		1212	257–411 calAD	None	

		1312	18–130 calAD	Possible tuberculous dactylitis	
Easington/Ganstead, Durham	Roman	25183	2 nd AD	Destructive lesions in T5–T10, L1–L3 and L5	Keefe and Holst, 2011
West Thurrock, Purfleet, Essex	Roman	10230	1 st AD	None	McKinley, 2007
		10287	1 st AD	Destructive lesions in T1, T6–8, T10, L5 and S1	
		10320	1 st AD	Destructive lesions in T7–8, T11	
		10333	1 st AD	Tuberculous septic arthritis of left elbow with ankylosis of radius and ulna and loss of most of the distal half of the humerus; periosteal new bone formation on left scapula, ulna, radius and humerus	
Ashchurch Bridge, Ashchurch, Gloucestershire	Roman	705	129–317 calAD	Visceral surface woven new bone formation on ribs	Holst, 2004
Cirencester, Gloucestershire	Roman	S		Lesions consistent with Pott's disease, with fusion of L1 and L2	Wells, 1982
Gambier Parry Lodge, Gloucester, Gloucestershire	Roman	500		Visceral surface woven new bone formation on ribs	Cameron and Roberts, 1984
		531		Visceral surface woven new bone formation on ribs	
		538		Visceral surface woven new bone formation on ribs	
		545	355–535 calAD	None	
Kingsholm, Gloucestershire	Roman	96	240–386 calAD	Visceral surface woven and/or lamellar new bone formation on ribs	Roberts, 1989

		131		Visceral surface woven new bone formation on ribs	
		236	259–425 calAD	Visceral surface woven new bone formation on ribs	
Wheatpieces, Tewkesbury, Gloucestershire	Roman	4	28–211 calAD	None	Holst, 2007
Victoria Road, Winchester, Hampshire	Roman	96		Visceral surface woven new bone formation on ribs	Ottaway et al., 2012
Baldock, Hertfordshire	Roman	7230	2–126 calAD	Destructive lesions in lower thoracic and lumbar vertebrae	McKinley, 1993
		7490		Destructive lesions in bodies of T11, L2 and L3, new bone formation on T10	
		7498		Destructive lesions in bodies of T9–T12 and L1–L3, with almost total collapse	
Newarke Street, Leicester, Leicestershire	Roman	427	late 4 th AD	Visceral surface new bone formation on ribs; destructive lesions in upper thoracic vertebrae	Wakely and Carter, 1996.
Ancaster, Lincolnshire	Roman	1	3 rd –4 th AD	New bone formation on the right auricular surface of the sacrum, right femoral head and right acetabulum with lesions consistent with TB; sinuses at the distal end of the right tibia and one tarsal, suggesting infection	Cox, 1989
		11	3 rd –4 th AD	Lesions consistent with Pott's disease, with fusion of T7–T10; T9 completely collapsed	

Horncastle, Lincolnshire	Roman	6	136–335 calAD	None	Caffell and Holst, 2008
		20	3 rd AD	None	
		45	3 rd AD	Calcified pleura	
		167	3 rd AD	Lytic lesions in vertebral bodies of T11–L1 and L4	
		186	3 rd AD	Lytic lesions in vertebral bodies of T11 and L1–L5	
		274	3 rd AD	Visceral surface woven new bone formation on ribs, lytic lesions in T6 and T11, widespread periostitis: woven and lamellar bone on T6, T7, scapulae, right humerus, radius and ulna, left ulna, all metacarpals, ilia, femora, tibiae and fibulae, calcanei and metatarsals	
Ashton, Northamptonshire	Roman	434	85–231 calAD	Septic arthritis of right wrist with affected distal radius, ulna, scaphoid and lunate and second metacarpal; septic arthritis of left ankle and affected talus, navicular and anterior calcaneus; woven and lamellar bone on the left tibia and fibula; possibly septic arthritis of right proximal fibula	Stirland and Waldron, 1990
		118	257–415 calAD	None	
		261	261–505 calAD	Destructive lesions in vertebral bodies of T5–L5,	

				with almost complete destruction of inferior body of T11; woven new bone formation on the neural arches of three thoracic vertebrae	
Water Lane, Towcester, Northamptonshire Queensford Mill, Oxfordshire	Roman	1	2 nd –4 th AD	None	Anderson et al., 2013
		151	4 th –early 5 th AD	None	Harman et al., 1978, 1981
	Roman	157	236–382 calAD	Lesions consistent with Pott's disease affecting T9–L3; destruction and collapse of T11 and T12 vertebral bodies	
Weston-super-Mare, Somerset	Roman	01		Lateral parts of vertebral bodies with sinuses in L1–L5; destructive lesion on left humeral head	Holst, 2010
3 Driffield Terrace, York, Yorkshire	Roman	13	late 2 nd –early 3 rd AD	Visceral surface woven and lamellar new bone formation on ribs; woven and lamellar bone on mandible; lamellar bone on femora, both first metatarsals and fifth right metatarsal	Caffell and Holst, 2012; Müldner et al., 2011
Heslington East, York, Yorkshire	Roman	229	302 ±39 AD	Destructive and proliferative lesions in L3–L5 and left sacro-iliac joint, the latter being fused; lamellar periosteal new bone formation on both tibiae	Holst, 2008; Neal and Roskams, 2012

Auldham, East Lothian	High/Late Medieval	43	1280–1394 calAD	Visceral surface woven new bone formation on ribs	Jennings, 2010; Lamb et al., 2012; Crone et al, forthcoming;
St Peter's Church, Leicester, Leicestershire	High Medieval	1390	1016–1155 calAD	Lytic lesions on the visceral surface of ribs; visceral surface woven and lamellar new bone formation on ribs	Jacklin, 2009
St Benet Sherehog, London, Greater London	Post Medieval	88	16 th –17 th AD	Visceral surface woven and lamellar new bone formation on ribs	Miles and White, 2008; WORD database, 2012
Manchester Hanging Ditch, Greater Manchester	Post Medieval	93	mid–18 th AD	Visceral surface woven and lamellar new bone formation on ribs	Archived Notes, Department of Archaeology, Durham University
Whitefriars, Norwich, Norfolk	Post Medieval	657	18 th –19 th AD	Visceral surface woven new bone formation on ribs	Caffell and Holst, 2006; Caffell and Clarke, 2011; Clarke, in prep; Caffell and Holst, in prep
		10466	18 th –19 th AD	Visceral surface woven and lamellar new bone formation on ribs; endocranial new bone formation; subtle patches of new woven bone throughout the skeleton	
St Peter's Collegiate Church, Wolverhampton, Staffordshire/West Midlands	Post Medieval	28	19 th AD	Visceral surface woven new bone formation on ribs	Adams and Colls, 2007
		62	19 th AD	Visceral surface woven to lamellar new bone formation on ribs, new bone formation on both	

St George's Crypt, Leeds, Yorkshire	Post Medieval	4005	mid-19 th AD	humeri, both scapulae and right radius (Disarticulated remains, more than one individual) visceral surface woven and lamellar new bone formation on ribs; woven new bone formation on two left and one right humeri, femora, tibiae and fibulae; woven new bone formation on one right scapula, one right ulna and one right radius as well as on one left calcaneum	Caffell and Holst, 2009
		4006	mid-19 th AD	visceral surface woven and lamellar new bone formation on ribs, woven new bone on one mandible and one calcaneum	
		5003	mid-19 th AD	None	
		<hr/>			
CONTINENTAL EUROPEAN SITES					
Slava Rusa (Ibida), Romania	Roman/ Early Medieval	M102	4 th –6 th AD	Visceral surface new bone formation on ribs; woven new bone formation on lumbar and thoracic vertebrae	Soficaru 2012
		M127	4 th –6 th AD	None	
Histria, Romania	Early Medieval	M66	6 th AD	Visceral surface new bone formation on ribs	Soficaru 2012
Obeliai, Lithuania	Early Medieval	143A	5 th –6 th AD	Lesions consistent with Pott's disease affecting L4	Česnys, 1988; Jankauskas, 1988;

		143B	5 th –6 th AD	and L5 None	Urbanavičius and Urbanavičienė, 1988; Prof Rimantas Jankauskas 2012, personal communication
Plinkaigalis, Lithuania	Early Medieval	150A	5 th –6 th AD	Lesions consistent with Pott's disease affecting T7– T12	Česnys, 1993; Jankauskas, 1993, 2002; Jankauskas and Kozlovskaya, 1999; Faerman and Jankauskas 2000; Prof Rimantas Jankauskas 2012, personal communication
Shchekavitsa, Kiev, Ukraine	High Medieval	8	late 10 th –12 th AD	Porous enlargements and new bone formations on the endocranial surface of the skull	Movchan et al., 1995/6; Dr Inna Potekhina and Dr Aleksandra Kozak 2012, personal communication
Naberezhno- Kreschatitskaya, Kiev, Ukraine	High Medieval	13	11 th –12 th AD	New bone formation on frontal bone, orbits, left greater trochanter and one left rib	Kozak, 2010; Kozak and Ivakin 2012; Dr Inna Potekhina and Dr Aleksandra Kozak 2012, personal communication
Saint Amé, Douai, France	Post Medieval	20	16–18 th AD	Visceral surface woven new bone formation on ribs	Dr William Devriendt 2008,

University of Göttingen, Germany	Post Medieval				personal communication
		13.k.36.1	19 th AD	Destruction of S1 and S2 and right sacro-iliac joint; new bone formation on right ilium	Prof Bernd Herrmann 2008, personal communication; Dr Birgit
		13.k.36.4	19 th AD	Destructive lesions in L4 and L5 vertebral bodies	Großkopf 2012, personal communication

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TABLE 1. Summary data for the samples analysed in this study

Site ID ¹	Period ²	Skeleton ID	Sample date ³	Reported age at death	Sex
BRITISH SITES					
Kempston, Bedfordshire	Roman	3902	3 rd –4 th AD	>45	Male
		3908	3 rd –4 th AD	36–45	Male
		3953	3 rd –4 th AD	18–25	Male
		3956	3 rd –4 th AD	36–45	Male
Addenbrookes, Cambridgeshire	Roman	3667	mid/late 1 st AD	18–25	Female
Duxford, Hinxton Road, Cambridgeshire	Roman	24	50 calBC–140 calAD	18–25	Female
Poundbury, Dorset	Roman	131		>45	Female
		228		Juvenile	?
		257		Juvenile	?
		1212	257–411 calAD	Adult	Female
		1312	18–130 calAD	Adult	Male
Easington/Ganstead, Durham	Roman	25183	2 nd AD	>45	Male
West Thurrock, Purfleet, Essex	Roman	10230	1 st AD	26–35	?
		10287	1 st AD	Young adult	Male
		10320	1 st AD	Mature adult	Male
		10333	1 st AD	Adult	Female
Ashchurch Bridge, Ashchurch, Gloucestershire	Roman	705	129–317 calAD	13–15	?
Cirencester, Gloucestershire	Roman	S		18–25	Male
Gambier Parry Lodge, Gloucester, Gloucestershire	Roman	500		>25	Male
		531		25–35	Female?
		538		8–9	?
		545	355–535 calAD	35–45	Female
Kingsholm, Gloucestershire	Roman	96	240–386 calAD	25–35	Female

		131		18–20	Female
		236	259–425 calAD	16	Male?
Wheatpieces, Tewkesbury, Gloucestershire	Roman	4	28–211 calAD	>45	Male
Victoria Road, Winchester, Hampshire	Roman	96		18–25	Female
Baldock, Hertfordshire	Roman	7230	2–126 calAD	26–35	Male
		7490		36–45	Male
		7498		26–35	Female
Newarke Street, Leicester, Leicestershire	Roman	427	late 4 th AD	18–25	Female
Ancaster, Lincolnshire	Roman	1	3 rd –4 th AD	18–25	Female
		11	3 rd –4 th AD	>45	Male
Horncastle, Lincolnshire	Roman	6	136–335 calAD	35–45	Male?
		20	3 rd AD	25–35	Male?
		45	3 rd AD	>45	Male?
		167	3 rd AD	35–45	Female?
		186	3 rd AD	35–45	Male
		274	3 rd AD	25–35	Female
		434	85–231 calAD	35–45	Male
Ashton, Northamptonshire	Roman	118	257–415 calAD	Adult	Female?
		261	261–505 calAD	Adult	Male
Water Lane, Towcester, Northamptonshire	Roman	1	2 nd –4 th AD	30–45	Male
Queensford Mill, Oxfordshire		151	4 th –early 5 th AD	36–45	Female
	Roman	157	236–382 calAD	>25	Female
Weston-super-Mare, Somerset	Roman	01		36–45	Male?
3 Driffield Terrace, York, Yorkshire	Roman	13	late 2 nd –early 3 rd AD	16–19	Male?
Heslington East, York, Yorkshire	Roman	229	302 ±39 AD	26–35	Male

Auldhame, East Lothian	High/Late Medieval	43	1280–1394 calAD	18–25	Male
St Peter’s Church, Leicester, Leicestershire	High Medieval	1390	1016–1155 calAD	16–18	?
St Benet Sherehog, London, Greater London	Post Medieval	88	16 th –17 th AD	>45	Male
Manchester Hanging Ditch, Greater Manchester	Post Medieval	93	mid–18 th AD	18–25	Male
Whitefriars, Norwich, Norfolk	Post Medieval	657	18 th –19 th AD	18–25	Female
		10466	18 th –19 th AD	17–18	Female?
St Peter’s Collegiate Church, Wolverhampton, Staffordshire/West Midlands	Post Medieval	28	19 th AD	26–35	Female
		62	19 th AD	>45	Female
St George’s Crypt, Leeds, Yorkshire	Post Medieval	4005	mid-19 th AD	16–18	?
		4006	mid-19 th AD	16–18	Female?
		5003	mid-19 th AD	>45	Female
CONTINEANTAL EUROPEAN SITES					
Slava Rusa (Ibida), Romania	Roman/Early Medieval	M102	4 th –6 th AD	25	Male
		M127	4 th –6 th AD	40	Male
Histria, Romania	Early Medieval	M66	6 th AD	60	Male
Obeliai, Lithuania	Early Medieval	143A	5 th –6 th AD	40–45	Male
		143B	5 th –6 th AD	Adult	Female
Plinkaigalis, Lithuania	Early Medieval	150A	5 th –6 th AD	25–30	Female
Shchekavitsa, Kiev, Ukraine	High Medieval	8	late 10 th –12 th AD	25–35	Female
Naberezhno-Kreschatitskaya, Kiev, Ukraine	High Medieval	13	11 th –12 th AD	35–45	Female
Saint Amé, Douai, France	Post Medieval	20	16–18 th AD	26–35	Male

University of Göttingen, Germany	Post Medieval	13.k.36.1	19 th AD	16–18	Male
		13.k.36.4	19 th AD	Adult	?

¹ For references to excavation and osteology reports, see Supporting Information.

² Definition of periods: Roman, 1st–4th centuries AD; Early Medieval 5th–10th centuries AD; High Medieval, 11th–13th centuries AD; Late Medieval, 14th– mid-16th centuries AD; Post Medieval, mid-16th–19th centuries AD.

³ ¹⁴C dates for individual samples are given when available, otherwise the site usage date is given.

⁴ For more complete information and images, see Supporting Information.

TABLE 2. Results of the DNA analyses

Sample ID	IS1081 detections ¹	IS6110 detections ²
SAMPLES IDENTIFIED AS CONTAINING MTBC aDNA		
Ashchurch 705 (rib)	– / +	– / 92 bp / 92 bp
Auldhame 43	+ / +	123 bp / 123 bp / 92 bp
Göttingen 13.k.36.1	+ / +	92 bp / 123 bp / n.d.
Saint Amé 20	+ / +	– / 123 bp / 92 bp
Shchekavits 8	+ / +	123 bp / 123 bp / n.d.
St George's Crypt 4006 (rib)	+ / +	123 bp / 123 bp / 123 bp
St George's Crypt 5003	+ / +	123 bp / 123 bp / n.d.
St Peter's Collegiate Church 28	+ / +	– / 123 bp ³ / –
St Peter's Collegiate Church 62 (rib)	+ / +	123 bp / 123 bp / 92 bp
St Peter's Church 1390 (rib)	+ / +	123 bp / 123 bp / 92 bp
Whitefriars 657 (tooth)	+ / +	n.d. / 123 bp ⁴ / n.d.
Whitefriars 10466	+ / +	123 bp / 123 bp / 123 bp
SAMPLES IDENTIFIED AS PROBABLY CONTAINING MTBC aDNA		
Ashchurch 705 (tooth)	n.d. / +	n.d. / 92 bp / n.d.
Ashton 118	– / +	– / 92 bp ⁵ / –
Ashton 261	– / –	– / 123 bp ⁶ / –
Horncastle 20	– / –	– / 123 bp ⁶ / n.d.
Horncastle 434	– / +	– / 123 bp ⁶ / n.d.
Kingsholm 96	– / +	92 bp ⁶ / – / –
Kingsholm 236 (rib)	– / +	– / 123 bp ⁶ / –
Obeliai 143A	– / +	– / 123 bp ⁶ / –
St George's Crypt 4006 (tooth)	n.d. / +	n.d. / 92 bp / n.d.
Water Lane 1 (femur)	– / +	– / 92 bp ⁵ / –
West Thurrock 10287	– / –	– / 123 bp ⁶ / n.d.
Wheatpieces 4	– / –	– / 92 bp ⁵ / –
SAMPLES IDENTIFIED AS POSSIBLY CONTAINING MTBC aDNA		
Ancaster 11	– / +	– / – / n.d.
Baldock 7230	– / +	– / – / n.d.
Göttingen 13.k.36.4	+ / +	– / – / n.d.

Kempston 3902	– / +	– / – / n.d.
Manchester Hanging Ditch 93	+ / –	– / – / n.d.
Naberezhno-Kreschatitskaya 13	+ / –	– / – / n.d.
Queensford Mill 157	– / +	– / – / n.d.
St Benet Sherehog 88	+ / +	– / – / n.d.
St George's Crypt 4005	+ / +	– / – / n.d.
Whitefriars 657 (rib)	+ / +	– / – / n.d.

SAMPLES IDENTIFIED AS NOT CONTAINING MTBC aDNA

3 Driffeld Terrace 13, Addenbrookes 3667, Ancaster 1, Baldock 7490 and 7498,
 Cirencester S, Duxford 24, Easington/Ganstead 25183, Gambier Parry Lodge 500, 531,
 538 and 545, Heslington East 229, Histria M66, Horncastle 6, 45, 167, 186 and 274,
 Kempston 3908, 3953 and 3956, Kingsholm 131 and 236 (tooth), Newarke Street 427,
 Obeliai 143B, Plinkaigalis 150A, Poundbury 131, 228, 257, 1212 and 1312, Queensford
 Mill 151, Slava Rusa M102 and M127, St Peter's Church 1390 (tooth), St Peter's
 Collegiate Church 62 (tooth), Victoria Road 96, Water Lane 1 (tooth), West Thurrock
 10230, 10320 and 10333, Weston-super-Mare 01

Key: +, positive result; –, negative result; n.d., not done.

¹ Results expressed as extraction by protocol I/extraction by protocol II, both performed in Manchester.

² '123 bp' indicates that a product of this size with the correct sequence was obtained after first-round PCR. '92 bp' indicates that detection was only after the second-round nested PCR. Results expressed as protocol I/protocol II/second extraction by protocol II performed in Madrid.

³ An additional Manchester extraction using protocol II gave a 92 bp amplification product.

⁴ An additional Manchester extraction using protocol II gave a 123 bp amplification product.

⁵ Sample gave the 92 bp amplification product after repeated PCR with the same extract.

⁶ Sample failed to give an amplification product after repeated PCR with the same extract.

TABLE 3. *Pathological changes in skeletons sampled and the presence of MTBC aDNA*

aDNA category	Sampled skeletal element			
	Vertebra from skeleton with TB specific lesions	Rib from skeleton with lesions (non-specific for TB)	Other affected part of skeleton with lesions (non-specific for TB)	Non-a skeleton (specific)
Definite MTBC aDNA	0	8	1	
Probable MTBC aDNA	2	2	1	
Possible MTBC aDNA	2	4	0	
No MTBC aDNA	7	13	4	
TOTAL	11	27	6	

Biomolecular Identification of Ancient *Mycobacterium tuberculosis* Complex DNA in Human Remains from Britain and Continental Europe

Romy Müller, Charlotte A. Roberts and Terence A. Brown

SUPPORTING INFORMATION

TABLE S1. Full description of skeletons sampled. (this file)

Fig. S1. Alignment of clones for the 123 bp and 92 bp products of IS6110 from samples identified to definitely contain MTBC aDNA. (Müller Fig. S1.pdf)

Fig. S2. Alignment of clones for the 123 bp and 92 bp products of IS6110 from samples identified to probably contain MTBC aDNA. (Müller Fig. S2.pdf)

Fig. S3. Images of pathological lesions displayed by the individuals investigated. (Müller Fig. S3.pdf)

TABLE S1. Full description of skeletons sampled.

Site ID	Period	Skeleton ID	Sample date	Parts of skeleton affected or possibly affected by TB	Reference
BRITISH SITES					
Kempston, Bedfordshire	Roman	3902	3 rd –4 th AD	None	Boylston and Roberts, 1996;
		3908	3 rd –4 th AD	Visceral surface woven new bone formation on ribs	Boylston et al., 2000
		3953	3 rd –4 th AD	Visceral surface woven new bone formation on ribs	
		3956	3 rd –4 th AD	Visceral surface woven new bone formation on ribs	
Addenbrookes, Cambridgeshire	Roman	3667	mid/late 1 st AD	Destructive lesions in T12 and three lumbar vertebrae (L1 and either L3 and L4, or L4 and L5), with collapse of two of the latter	Dodwell, 2008
Duxford, Hinxton Road, Cambridgeshire	Roman	24	50 calBC–140 calAD	Destructive lesions in lumbar vertebrae	Lyons, 2011
Poundbury, Dorset	Roman	131		Lesions consistent with Pott's disease affecting three vertebral bodies with collapsing and anterior bony ankylosis	Farwell and Molleson 1993; Lewis 2011
		228		Lytic lesions and visceral surface woven new bone formation on ribs	
		257		Visceral surface woven new bone formation on ribs	

		1212	257–411 calAD	None	
		1312	18–130 calAD	Possible tuberculous dactylitis	
Easington/Ganstead, Durham	Roman	25183	2 nd AD	Destructive lesions in T5–T10, L1–L3 and L5	Keefe and Holst, 2011
West Thurrock, Purfleet, Essex	Roman	10230	1 st AD	None	McKinley, 2007
		10287	1 st AD	Destructive lesions in T1, T6–8, T10, L5 and S1	
		10320	1 st AD	Destructive lesions in T7–8, T11	
		10333	1 st AD	Tuberculous septic arthritis of left elbow with ankylosis of radius and ulna and loss of most of the distal half of the humerus; periosteal new bone formation on left scapula, ulna, radius and humerus	
Ashchurch Bridge, Ashchurch, Gloucestershire	Roman	705	129–317 calAD	Visceral surface woven new bone formation on ribs	Holst, 2004
Cirencester, Gloucestershire	Roman	S		Lesions consistent with Pott's disease, with fusion of L1 and L2	Wells, 1982
Gambier Parry Lodge, Gloucester, Gloucestershire	Roman	500		Visceral surface woven new bone formation on ribs	Cameron and Roberts, 1984
		531		Visceral surface woven new bone formation on ribs	
		538		Visceral surface woven new bone formation on ribs	
		545	355–535 calAD	None	
Kingsholm, Gloucestershire	Roman	96	240–386 calAD	Visceral surface woven and/or lamellar new bone	Roberts, 1989

				formation on ribs	
		131		Visceral surface woven new bone formation on ribs	
		236	259–425 calAD	Visceral surface woven new bone formation on ribs	
Wheatpieces, Tewkesbury, Gloucestershire Victoria Road, Winchester, Hampshire Baldock, Hertfordshire	Roman	4	28–211 calAD	None	Holst, 2007
	Roman	96		Visceral surface woven new bone formation on ribs	Ottaway et al., 2012
	Roman	7230	2–126 calAD	Destructive lesions in lower thoracic and lumbar vertebrae	McKinley, 1993
		7490		Destructive lesions in bodies of T11, L2 and L3, new bone formation on T10	
		7498		Destructive lesions in bodies of T9–T12 and L1–L3, with almost total collapse	
Newarke Street, Leicester, Leicestershire	Roman	427	late 4 th AD	Visceral surface new bone formation on ribs; destructive lesions in upper thoracic vertebrae	Wakely and Carter, 1996.
Ancaster, Lincolnshire	Roman	1	3 rd –4 th AD	New bone formation on the right auricular surface of the sacrum, right femoral head and right acetabulum with lesions consistent with TB; sinuses at the distal end of the right tibia and one tarsal, suggesting infection	Cox, 1989

Horncastle, Lincolnshire	Roman	11	3 rd –4 th AD	Lesions consistent with Pott's disease, with fusion of T7–T10; T9 completely collapsed	Caffell and Holst, 2008
		6	136–335 calAD	None	
		20	3 rd AD	None	
		45	3 rd AD	Calcified pleura	
		167	3 rd AD	Lytic lesions in vertebral bodies of T11–L1 and L4	
		186	3 rd AD	Lytic lesions in vertebral bodies of T11 and L1–L5	
		274	3 rd AD	Visceral surface woven new bone formation on ribs, lytic lesions in T6 and T11, widespread periostitis: woven and lamellar bone on T6, T7, scapulae, right humerus, radius and ulna, left ulna, all metacarpals, ilia, femora, tibiae and fibulae, calcanei and metatarsals	
		434	85–231 calAD	Septic arthritis of right wrist with affected distal radius, ulna, scaphoid and lunate and second metacarpal; septic arthritis of left ankle and affected talus, navicular and anterior calcaneus; woven and lamellar bone on the left tibia and fibula; possibly septic arthritis of right proximal fibula	

Ashton, Northamptonshire	Roman	118	257–415 calAD	None	Stirland and Waldron, 1990
		261	261–505 calAD	Destructive lesions in vertebral bodies of T5–L5, with almost complete destruction of inferior body of T11; woven new bone formation on the neural arches of three thoracic vertebrae	
Water Lane, Towcester, Northamptonshire Queensford Mill, Oxfordshire	Roman	1	2 nd –4 th AD	None	Anderson et al., 2013
		151	4 th –early 5 th AD	None	Harman et al., 1978, 1981
	Roman	157	236–382 calAD	Lesions consistent with Pott's disease affecting T9–L3; destruction and collapse of T11 and T12 vertebral bodies	
Weston-super-Mare, Somerset	Roman	01		Lateral parts of vertebral bodies with sinuses in L1–L5; destructive lesion on left humeral head	Holst, 2010
3 Driffield Terrace, York, Yorkshire	Roman	13	late 2 nd –early 3 rd AD	Visceral surface woven and lamellar new bone formation on ribs; woven and lamellar bone on mandible; lamellar bone on femora, both first metatarsals and fifth right metatarsal	Caffell and Holst, 2012; Müldner et al., 2011
Heslington East, York, Yorkshire	Roman	229	302 ±39 AD	Destructive and proliferative lesions in L3–L5 and left sacro-iliac joint, the latter being fused; lamellar periosteal new bone formation on both tibiae	Holst, 2008; Neal and Roskams, 2012

Auldham, East Lothian	High/Late Medieval	43	1280–1394 calAD	Visceral surface woven new bone formation on ribs	Jennings, 2010; Lamb et al., 2012; Crone et al, forthcoming;
St Peter's Church, Leicester, Leicestershire	High Medieval	1390	1016–1155 calAD	Lytic lesions on the visceral surface of ribs; visceral surface woven and lamellar new bone formation on ribs	Jacklin, 2009
St Benet Sherehog, London, Greater London	Post Medieval	88	16 th –17 th AD	Visceral surface woven and lamellar new bone formation on ribs	Miles and White, 2008; WORD database, 2012
Manchester Hanging Ditch, Greater Manchester	Post Medieval	93	mid–18 th AD	Visceral surface woven and lamellar new bone formation on ribs	Archived Notes, Department of Archaeology, Durham University
Whitefriars, Norwich, Norfolk	Post Medieval	657	18 th –19 th AD	Visceral surface woven new bone formation on ribs	Caffell and Holst, 2006; Caffell and Clarke, 2011; Clarke, in prep; Caffell and Holst, in prep
		10466	18 th –19 th AD	Visceral surface woven and lamellar new bone formation on ribs; endocranial new bone formation; subtle patches of new woven bone throughout the skeleton	
St Peter's Collegiate Church, Wolverhampton, Staffordshire/West Midlands	Post Medieval	28	19 th AD	Visceral surface woven new bone formation on ribs	Adams and Colls, 2007
		62	19 th AD	Visceral surface woven to lamellar new bone formation on ribs, new bone formation on both	

St George's Crypt, Leeds, Yorkshire	Post Medieval	4005	mid-19 th AD	humeri, both scapulae and right radius (Disarticulated remains, more than one individual) visceral surface woven and lamellar new bone formation on ribs; woven new bone formation on two left and one right humeri, femora, tibiae and fibulae; woven new bone formation on one right scapula, one right ulna and one right radius as well as on one left calcaneum	Caffell and Holst, 2009
		4006	mid-19 th AD	visceral surface woven and lamellar new bone formation on ribs, woven new bone on one mandible and one calcaneum	
		5003	mid-19 th AD	None	
		<hr/>			
CONTINENTAL EUROPEAN SITES					
Slava Rusa (Ibida), Romania	Roman/ Early Medieval	M102	4 th –6 th AD	Visceral surface new bone formation on ribs; woven new bone formation on lumbar and thoracic vertebrae	Soficaru 2012
		M127	4 th –6 th AD	None	
Histria, Romania	Early Medieval	M66	6 th AD	Visceral surface new bone formation on ribs	Soficaru 2012
Obeliai, Lithuania	Early Medieval	143A	5 th –6 th AD	Lesions consistent with Pott's disease affecting L4	Česnys, 1988; Jankauskas, 1988;

		143B	5 th –6 th AD	and L5 None	Urbanavičius and Urbanavičienė, 1988; Prof Rimantas Jankauskas 2012, personal communication
Plinkaigalis, Lithuania	Early Medieval	150A	5 th –6 th AD	Lesions consistent with Pott's disease affecting T7– T12	Česnys, 1993; Jankauskas, 1993, 2002; Jankauskas and Kozlovskaya, 1999; Faerman and Jankauskas 2000; Prof Rimantas Jankauskas 2012, personal communication
Shchekavitsa, Kiev, Ukraine	High Medieval	8	late 10 th –12 th AD	Porous enlargements and new bone formations on the endocranial surface of the skull	Movchan et al., 1995/6; Dr Inna Potekhina and Dr Aleksandra Kozak 2012, personal communication
Naberezhno- Kreschatitskaya, Kiev, Ukraine	High Medieval	13	11 th –12 th AD	New bone formation on frontal bone, orbits, left greater trochanter and one left rib	Kozak, 2010; Kozak and Ivakin 2012; Dr Inna Potekhina and Dr Aleksandra Kozak 2012, personal communication
Saint Amé, Douai, France	Post Medieval	20	16–18 th AD	Visceral surface woven new bone formation on ribs	Dr William Devriendt 2008,

personal communication

University of
Göttingen, Germany

Post
Medieval

13.k.36.1 19th AD

Destruction of S1 and S2 and right sacro-iliac joint;
new bone formation on right ilium

Prof Bernd Herrmann 2008,
personal communication; Dr Birgit

13.k.36.4 19th AD

Destructive lesions in L4 and L5 vertebral bodies

Großkopf 2012, personal
communication

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TABLE 1. Summary data for the samples analysed in this study

Site ID ¹	Period ²	Skeleton ID	Sample date ³	Reported age at death	Sex	Elements showing lesions ⁴	Sampled element
BRITISH SITES							
Kempston, Bedfordshire	Roman	3902	3 rd –4 th AD	>45	Male	None	Femur
		3908	3 rd –4 th AD	36–45	Male	Ribs	Rib
		3953	3 rd –4 th AD	18–25	Male	Ribs	Rib
		3956	3 rd –4 th AD	36–45	Male	Ribs	Rib
Addenbrookes, Cambridgeshire	Roman	3667	mid/late 1 st AD	18–25	Female	Vertebrae	Rib
Duxford, Hinxton Road, Cambridgeshire	Roman	24	50 calBC–140 calAD	18–25	Female	Vertebrae	Rib
Poundbury, Dorset	Roman	131		>45	Female	Vertebrae	Femur
		228		Juvenile	?	Ribs	Rib
		257		Juvenile	?	Ribs	Rib
		1212	257–411 calAD	Adult	Female	None	Fibula
		1312	18–130 calAD	Adult	Male	Digits	Rib
Easington/Ganstead, Durham	Roman	25183	2 nd AD	>45	Male	Vertebrae	Vertebra
West Thurrock, Purfleet, Essex	Roman	10230	1 st AD	26–35	?	None	Tibia
		10287	1 st AD	Young adult	Male	Vertebrae	Vertebra
		10320	1 st AD	Mature adult	Male	Vertebrae	Vertebra

		10333	1 st AD	Adult	Female	Elbow, radius, ulna, humerus, scapula	Femur
Ashchurch Bridge, Ashchurch, Gloucestershire	Roman	705	129–317 calAD	13–15	?	Ribs	Rib, tooth
Cirencester, Gloucestershire	Roman	S		18–25	Male	Vertebrae	Humerus
Gambier Parry Lodge, Gloucester, Gloucestershire	Roman	500		>25	Male	Ribs	Rib
		531		25–35	Female?	Ribs	Rib
		538		8–9	?	Ribs	Rib
		545	355–535 calAD	35–45	Female	None	Rib
Kingsholm, Gloucestershire	Roman	96	240–386 calAD	25–35	Female	Ribs	Rib
		131		18–20	Female	Ribs	Rib
		236	259–425 calAD	16	Male?	Ribs	Rib, tooth
Wheatpieces, Tewkesbury, Gloucestershire	Roman	4	28–211 calAD	>45	Male	None	Femur
Victoria Road, Winchester, Hampshire	Roman	96		18–25	Female	Ribs	Rib
Baldock, Hertfordshire	Roman	7230	2–126 calAD	26–35	Male	Vertebrae	Vertebra
		7490		36–45	Male	Vertebrae	Femur
		7498		26–35	Female	Vertebrae	Vertebra
Newarke Street, Leicester, Leicestershire	Roman	427	late 4 th AD	18–25	Female	Ribs, vertebrae	Rib
Ancaster, Lincolnshire	Roman	1	3 rd –4 th AD	18–25	Female	Sacrum, femur, tibia, tarsus	Femur

		11	3 rd –4 th AD	>45	Male	Vertebrae	Tibia
Horncastle, Lincolnshire	Roman	6	136–335 calAD	35–45	Male?	None	Tibia
		20	3 rd AD	25–35	Male?	None	Radius
		45	3 rd AD	>45	Male?	Calcified pleura	Pleura
		167	3 rd AD	35–45	Female?	Vertebrae	Vertebra
		186	3 rd AD	35–45	Male	Vertebrae	Vertebra
		274	3 rd AD	25–35	Female	Many areas	Vertebra
		434	85–231 calAD	35–45	Male	Many areas	Radius
Ashton, Northamptonshire	Roman	118	257–415 calAD	Adult	Female?	None	Femur
		261	261–505 calAD	Adult	Male	Vertebrae	Vertebra
Water Lane, Towcester, Northamptonshire	Roman	1	2 nd –4 th AD	30–45	Male	None	Femur, tooth
Queensford Mill, Oxfordshire		151	4 th –early 5 th AD	36–45	Female	None	Humerus
	Roman	157	236–382 calAD	>25	Female	Vertebrae	Femur
Weston-super-Mare, Somerset	Roman	01		36–45	Male?	Vertebrae, humerus	Vertebra
3 Driffield Terrace, York, Yorkshire	Roman	13	late 2 nd –early 3 rd AD	16–19	Male?	Ribs, mandible, femora, metatarsals	Rib
Heslington East, York, Yorkshire	Roman	229	302 ±39 AD	26–35	Male	Vertebrae, sacro- iliac joint, tibia	Vertebra
Auldhame, East Lothian	High/Late Medieval	43	1280–1394 calAD	18–25	Male	Ribs	Rib

St Peter's Church, Leicester, Leicestershire	High Medieval	1390	1016–1155 calAD	16–18	?	Ribs	Rib, tooth
St Benet Sherehog, London, Greater London	Post Medieval	88	16 th –17 th AD	>45	Male	Ribs	Femur
Manchester Hanging Ditch, Greater Manchester	Post Medieval	93	mid–18 th AD	18–25	Male	Ribs	Rib
Whitefriars, Norwich, Norfolk	Post Medieval	657	18 th –19 th AD	18–25	Female	Ribs	Rib, tooth
		10466	18 th –19 th AD	17–18	Female?	Ribs, endocranium	Rib
St Peter's Collegiate Church, Wolverhampton, Staffordshire/West Midlands	Post Medieval	28	19 th AD	26–35	Female	Ribs	Rib
		62	19 th AD	>45	Female	Ribs, humeri, scapulae, radius	Rib, tooth
St George's Crypt, Leeds, Yorkshire	Post Medieval	4005	mid-19 th AD	16–18	?	Many areas	Rib
		4006	mid-19 th AD	16–18	Female?	Ribs, mandible, calcaneus	Rib, tooth
		5003	mid-19 th AD	>45	Female	None	Rib

CONTINEANTAL EUROPEAN SITES

Slava Rusa (Ibida), Romania	Roman/Early Medieval	M102	4 th –6 th AD	25	Male	Ribs, vertebrae	Vertebra
		M127	4 th –6 th AD	40	Male	None	Humerus
Histria, Romania	Early Medieval	M66	6 th AD	60	Male	Ribs	Rib
Obeliai, Lithuania	Early Medieval	143A	5 th –6 th AD	40–45	Male	Vertebrae	Tibia

		143B	5 th –6 th AD	Adult	Female	None	Tibia
Plinkaigalis, Lithuania	Early Medieval	150A	5 th –6 th AD	25–30	Female	Vertebrae	Tibia
Shchekavitsa, Kiev, Ukraine	High Medieval	8	late 10 th –12 th AD	25–35	Female	Endocranium	Femur
Naberezhno-Kreschatitskaya, Kiev, Ukraine	High Medieval	13	11 th –12 th AD	35–45	Female	Skull, humerus, rib	Rib
Saint Amé, Douai, France	Post Medieval	20	16–18 th AD	26–35	Male	Ribs	Rib
University of Göttingen, Germany	Post Medieval	13.k.36.1	19 th AD	16–18	Male	Vertebrae, sacro-iliac joint, ilium	Ilium
		13.k.36.4	19 th AD	Adult	?	Vertebrae	Vertebra

¹ For references to excavation and osteology reports, see Supporting Information.

² Definition of periods: Roman, 1st–4th centuries AD; Early Medieval 5th–10th centuries AD; High Medieval, 11th–13th centuries AD; Late Medieval, 14th–mid-16th centuries AD; Post Medieval, mid-16th–19th centuries AD.

³ ¹⁴C dates for individual samples are given when available, otherwise the site usage date is given.

⁴ For more complete information and images, see Supporting Information.

TABLE 2. Results of the DNA analyses

Sample ID	IS1081 detections ¹	IS6110 detections ²
SAMPLES IDENTIFIED AS CONTAINING MTBC aDNA		
Ashchurch 705 (rib)	– / +	– / 92 bp / 92 bp
Auldhame 43	+ / +	123 bp / 123 bp / 92 bp
Göttingen 13.k.36.1	+ / +	92 bp / 123 bp / n.d.
Saint Amé 20	+ / +	– / 123 bp / 92 bp
Shchekavits 8	+ / +	123 bp / 123 bp / n.d.
St George's Crypt 4006 (rib)	+ / +	123 bp / 123 bp / 123 bp
St George's Crypt 5003	+ / +	123 bp / 123 bp / n.d.
St Peter's Collegiate Church 28	+ / +	– / 123 bp ³ / –
St Peter's Collegiate Church 62 (rib)	+ / +	123 bp / 123 bp / 92 bp
St Peter's Church 1390 (rib)	+ / +	123 bp / 123 bp / 92 bp
Whitefriars 657 (tooth)	+ / +	n.d. / 123 bp ⁴ / n.d.
Whitefriars 10466	+ / +	123 bp / 123 bp / 123 bp
SAMPLES IDENTIFIED AS PROBABLY CONTAINING MTBC aDNA		
Ashchurch 705 (tooth)	n.d. / +	n.d. / 92 bp / n.d.
Ashton 118	– / +	– / 92 bp ⁵ / –
Ashton 261	– / –	– / 123 bp ⁶ / –
Horncastle 20	– / –	– / 123 bp ⁶ / n.d.
Horncastle 434	– / +	– / 123 bp ⁶ / n.d.
Kingsholm 96	– / +	92 bp ⁶ / – / –
Kingsholm 236 (rib)	– / +	– / 123 bp ⁶ / –
Obelgai 143A	– / +	– / 123 bp ⁶ / –
St George's Crypt 4006 (tooth)	n.d. / +	n.d. / 92 bp / n.d.
Water Lane 1 (femur)	– / +	– / 92 bp ⁵ / –
West Thurrock 10287	– / –	– / 123 bp ⁶ / n.d.
Wheatpieces 4	– / –	– / 92 bp ⁵ / –
SAMPLES IDENTIFIED AS POSSIBLY CONTAINING MTBC aDNA		
Ancaster 11	– / +	– / – / n.d.
Baldock 7230	– / +	– / – / n.d.
Göttingen 13.k.36.4	+ / +	– / – / n.d.

Kempston 3902	– / +	– / – / n.d.
Manchester Hanging Ditch 93	+ / –	– / – / n.d.
Naberezhno-Kreschatitskaya 13	+ / –	– / – / n.d.
Queensford Mill 157	– / +	– / – / n.d.
St Benet Sherehog 88	+ / +	– / – / n.d.
St George's Crypt 4005	+ / +	– / – / n.d.
Whitefriars 657 (rib)	+ / +	– / – / n.d.

SAMPLES IDENTIFIED AS NOT CONTAINING MTBC aDNA

3 Driffeld Terrace 13, Addenbrookes 3667, Ancaster 1, Baldock 7490 and 7498, Cirencester S, Duxford 24, Easington/Ganstead 25183, Gambier Parry Lodge 500, 531, 538 and 545, Heslington East 229, Histria M66, Horncastle 6, 45, 167, 186 and 274, Kempston 3908, 3953 and 3956, Kingsholm 131 and 236 (tooth), Newarke Street 427, Obeliai 143B, Plinkaigalis 150A, Poundbury 131, 228, 257, 1212 and 1312, Queensford Mill 151, Slava Rusa M102 and M127, St Peter's Church 1390 (tooth), St Peter's Collegiate Church 62 (tooth), Victoria Road 96, Water Lane 1 (tooth), West Thurrock 10230, 10320 and 10333, Weston-super-Mare 01

Key: +, positive result; –, negative result; n.d., not done.

¹ Results expressed as extraction by protocol I/extraction by protocol II, both performed in Manchester.

² '123 bp' indicates that a product of this size with the correct sequence was obtained after first-round PCR. '92 bp' indicates that detection was only after the second-round nested PCR. Results expressed as protocol I/protocol II/second extraction by protocol II performed in Madrid.

³ An additional Manchester extraction using protocol II gave a 92 bp amplification product.

⁴ An additional Manchester extraction using protocol II gave a 123 bp amplification product.

⁵ Sample gave the 92 bp amplification product after repeated PCR with the same extract.

⁶ Sample failed to give an amplification product after repeated PCR with the same extract.

TABLE 3. *Pathological changes in skeletons sampled and the presence of MTBC aDNA*

aDNA category	Sampled skeletal element				
	Vertebra from skeleton with TB specific lesions	Rib from skeleton with lesions (non-specific for TB)	Other affected part of skeleton with lesions (non-specific for TB)	Non-affected part of skeleton with lesions (specific or non-specific for TB)	Skeleton with no lesions
Definite MTBC aDNA	0	8	1	2	1
Probable MTBC aDNA	2	2	1	3	4
Possible MTBC aDNA	2	4	0	3	1
No MTBC aDNA	7	13	4	11	8
TOTAL	11	27	6	19	14

Supporting information for Muller et al.: Biomolecular Identification of Ancient *Mycobacterium tuberculosis* Complex DNA in Human Remains from Britain and Continental Europe

Figure S1. Alignment of clones for the 123 bp (longer sequence) and 92 bp product (shorter sequence) of IS6110 from samples identified to definitely contain MTBC aDNA. Primers are removed. The reference sequence for IS6110 is given at the top. Differences to the reference sequence, most likely representing damage-derived miscoding lesions or PCR errors (Brotherton et al., 2007; Gilbert et al., 2007), are highlighted in colour. Numbers 1–4 following the sample ID denote the respective extraction (1, 1st Manchester extraction protocol I; 2, 2nd Manchester extraction protocol II; 3, Madrid extraction protocol II; 4, additional Manchester extraction protocol II). Letters a–f indicate the respective clone obtained for each amplification.

IS6110-reference	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
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ASHCHURCH 705-2b	A	A	C	C	G	G	C	T	G	G	G	T	A	G	C	A	G	A	C	T	C	A	C	C	T	A	T	G	T	G	T	C	G	A	C	T	G	G	G	C	A	G	G	G	T	T	C	G	C	T	A	C	G	T	G	G	C	T	T	T	G	T	C	A																																				
ASHCHURCH 705-2c	A	A	C	C	G	G	C	T	G	G	G	T	A	G	C	A	G	A	C	T	C	A	C	C	T	A	T	G	T	G	T	C	G	A	C	T	G	G	G	C	A	G	G	G	T	T	C	G	C	T	A	C	G	T	G	G	C	T	T	T	G	T	C	A																																				
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Literature Cited

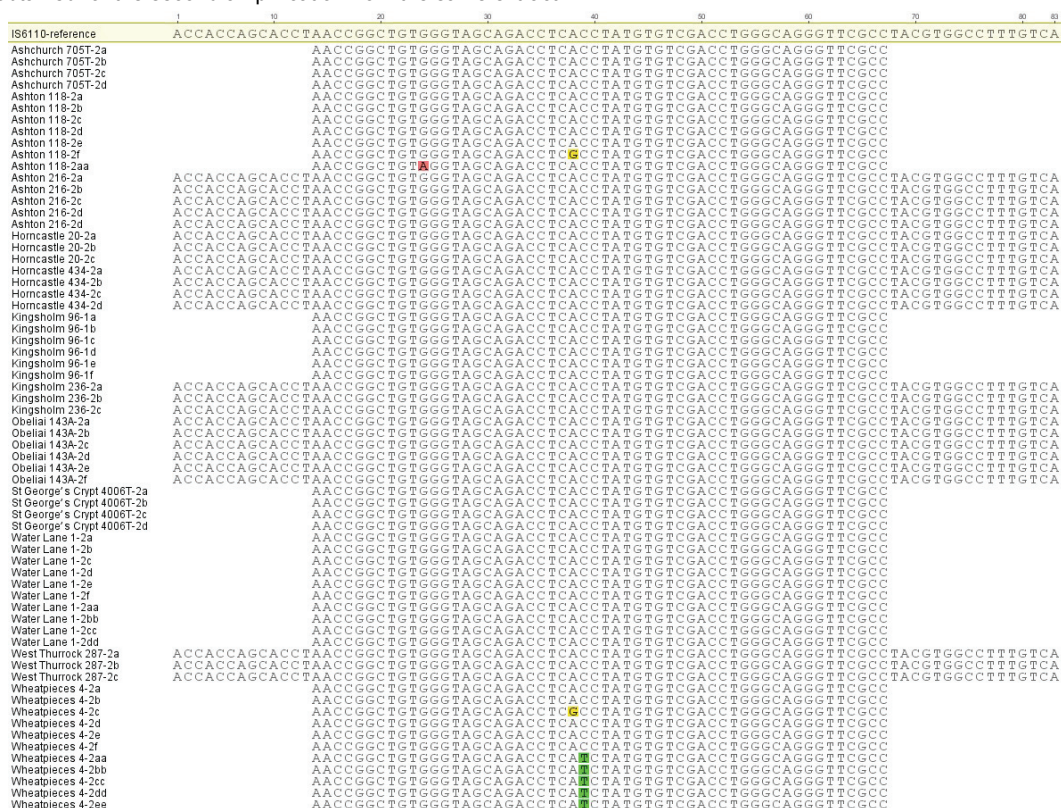
- Brotherton P, Endicott P, Sanchez JJ, Beaumont M, Barnett R, Austin J, Cooper A. 2007. Novel high-resolution characterization of ancient DNA reveals C > U-type base modification events as the sole cause of post mortem miscoding lesions. *Nucleic Acids Res* 35(17):5717–5728.
- Gilbert MTP, Binladen J, Miller W, Wiuf C, Willerslev E, Poinar H, Carlson JE, Leebens-Mack JH, Schuster SC. 2007. Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. *Nucleic Acids Res* 35(1):1–10.

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Complex DNA in Human Remains from Britain and Continental Europe

Figure S2. Alignment of clones for the 123 bp (longer sequence) and 92 bp product (shorter sequence) of IS6110 from samples identified to probably contain MTBC aDNA. Primer sites are removed. The reference sequence for IS6110 is given at the top. Differences to the reference sequence, most likely representing damage-derived miscoding lesions or PCR errors (Brotherton et al., 2007; Gilbert et al., 2007), are highlighted in colour. Numbers 1–4 following the sample

ID denote the respective extraction (1, 1st Manchester extraction protocol I; 2, 2nd Manchester extraction protocol II).

Letters a–f indicate the respective clone obtained for each amplification and letters aa–ee denote the respective clone obtained for the second amplification from the same extract.



Literature Cited

Brotherton P, Endicott P, Sanchez JJ, Beaumont M, Barnett R, Austin J, Cooper A. 2007. Novel high-resolution characterization of ancient DNA reveals C > U-type base modification events as the sole cause of post mortem miscoding lesions. *Nucleic Acids Res* 35(17):5717–5728.

Gilbert MTP, Binladen J, Miller W, Wiuf C, Willerslev E, Poinar H, Carlson JE, Leebens-Mack JH, Schuster SC. 2007. Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. *Nucleic Acids Res* 35(1):1–10.

Figures S3. Images of pathological lesions displayed by the individuals investigated (for those where a photograph was available). Lesions are highlighted by arrows or circles.

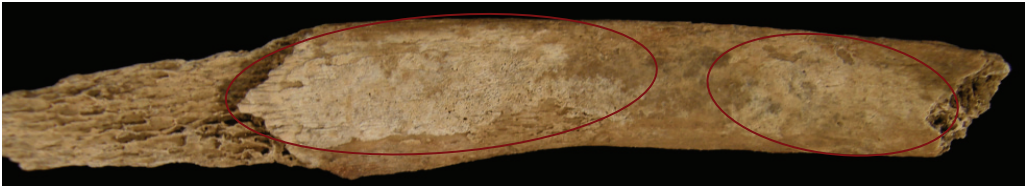


Figure S3-1. Visceral surface woven new bone formation on rib fragment of Kempston 3908.



Figure S3-2. Visceral surface woven new bone formation on rib fragments of Kempston 3953.



Figure S3-3. Visceral surface woven new bone formation on rib fragments of Kempston 3956.



Figure S3-4. Destructive lesions in lower thoracic and lumbar vertebrae of Addenbrookes 3667.



Figure S3-5. Destructive lesions in lumbar vertebrae of Duxford, Hinxton Road 24.



Figure S3-6. Destructive lesions in T6–T10 of West Thurrock 10287.

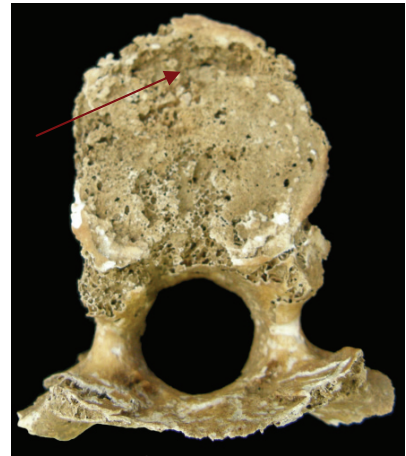


Figure S3-7. Destructive lesions in T8 of West Thurrock 10320.



Figure S3-8. Probable septic arthritis of left elbow of West Thurrock 10333, possibly caused by TB.

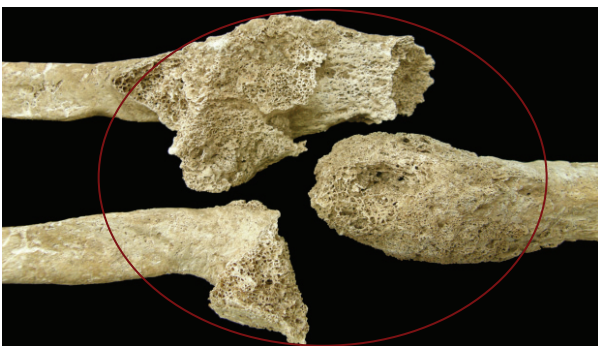


Figure S3-9. Close-up of left elbow of West Thurrock 10333 showing joint destruction and periosteal new bone formation on humerus, radius and ulna.



Figure S3-10. Visceral surface woven new bone formation on rib fragments of Ashchurch 705.



Figures S3-11 and S3-12. Destructive lesions in Cirencester S consistent with Pott's disease, with fusion of L1 and L2 (right and left sides).



Figure S3-13. Visceral surface woven new bone formation on rib fragment of Gambier Parry Lodge 500.

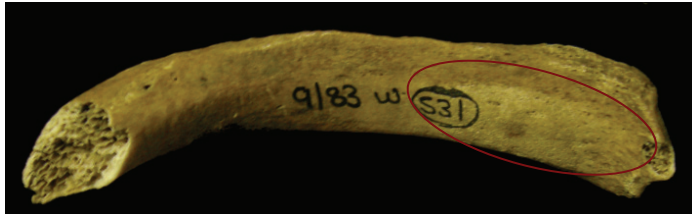


Figure S3-14. Visceral surface woven new bone formation on rib fragment of Gambier Parry Lodge 531.

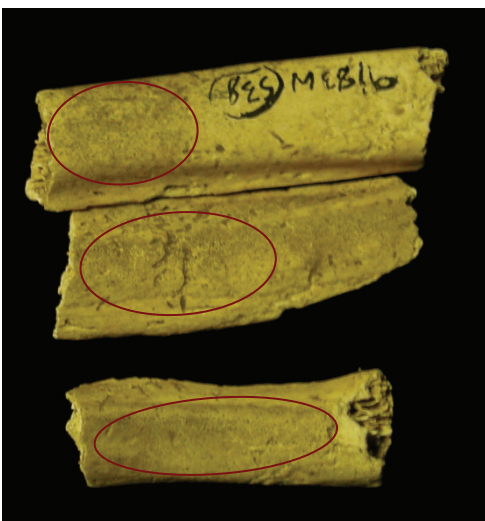


Figure S3-15. Visceral surface woven new bone formation on rib fragments of Gambier Parry Lodge 538.



Figure S3-16. Visceral surface woven and lamellar new bone formation on rib fragments of Kingsholm 96.



Figures S3-17. Visceral surface woven new bone formation on ribs of Kingsholm 131.



Figures S3-18. Visceral surface woven new bone formation on ribs of Kingsholm 236.



Figure S3-19. Visceral surface woven new bone formation on ribs of Victoria Road 96.

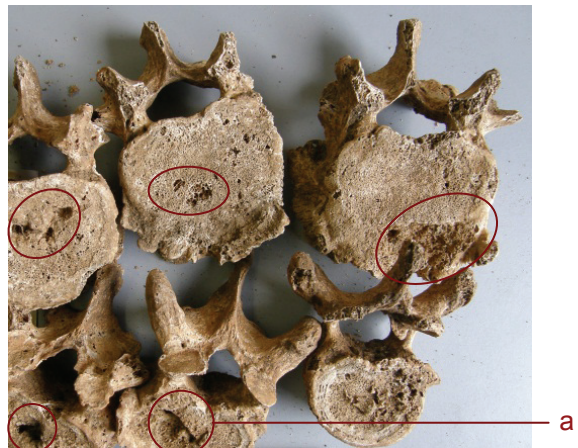


Figure S3-20. Destructive lesions in lower thoracic and lumbar vertebrae of Baldock 7230.



Figure S3-21. Close-up of destructive lesion (a) in lumbar vertebra of Baldock 7230.



Figure S3-22. Destructive lesions in vertebral bodies of thoracic vertebrae of Baldock 7498.



Figure S3-23. Right femoral head of Ancaster 1 with lesions possibly associated with TB.

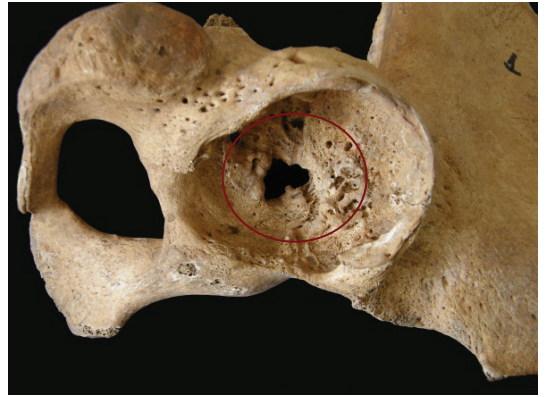


Figure S3-24. Right acetabulum of Ancaster 1 with lesions possibly associated with TB.



Figure S3-25. Destructive lesions on the right innominate bone of Ancaster 1, possibly associated with TB.



Figure S3-26. Sinuses at the distal end of the right tibia of Ancaster 1, possibly associated with TB in the joint.



Figure S3-27. Ancaster 11. Compression fracture of vertebrae and fusion possibly relating to Pott's disease of the spine.

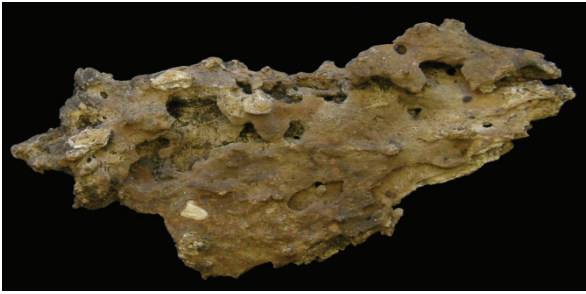


Figure S3-28. Calcified pleura (66mm x 26mm x 8mm) from Horncastle 45.

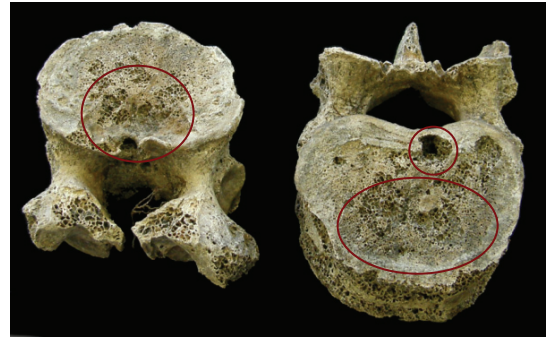


Figure S3-29. Lytic lesions in vertebral bodies of T11 and T12 of Horncastle 167.

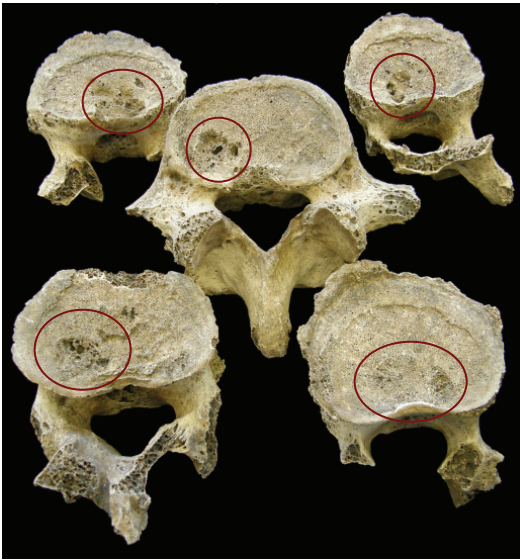


Figure S3-30. Lytic lesions in vertebral bodies of T11, T12 And L3–L5 of Horncastle 186.



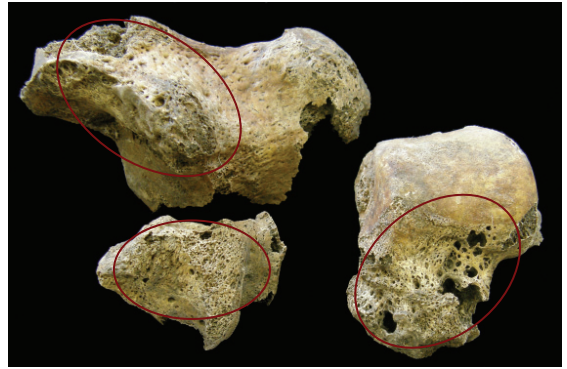
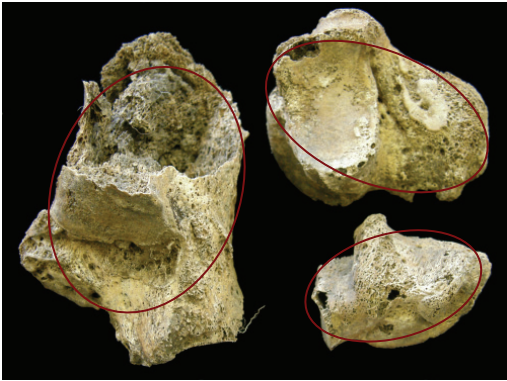
Figure S3-31. Lytic lesion in T11 of Horncastle 274.



Figure S3-32. Visceral surface woven new bone formation on rib fragments of Horncastle 274.



Figure S3-33. Probable septic arthritis (possibly due to TB) of the right wrist of Horncastle 434 with affected distal radius and ulna, scaphoid and lunate and second metacarpal.



Figures S3-34 and S3-35. Probable septic arthritis (possibly due to TB) of left ankle of Horncastle 434 with affected talus, navicular and calcaneus.

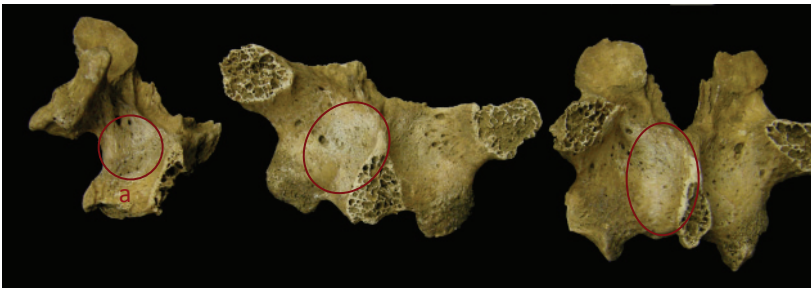


Figure S3-36. Woven New bone formation on the neural arches of three thoracic vertebrae of Ashton 261.

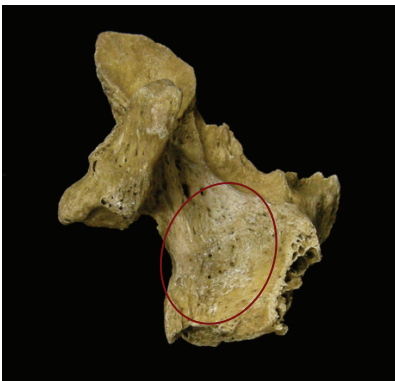


Figure S3-37. Close-up of the new bone formation on the neural arch of a thoracic vertebra of Ashton 261 (a).



Figure S3-38. Destructive lesions and fusion of lumbar vertebrae of Heslington East 229.



Figure S3-39. Visceral surface woven new bone formation on rib fragment of Auldham 43.

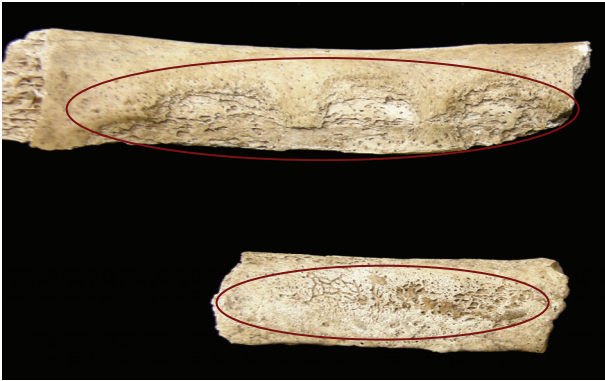


Figure S3-40. Rib fragments of St Peter's Church, Leicester 1390, displaying lytic lesions on their visceral surfaces as well as visceral surface woven and lamellar new bone formation.



Figure S3-41. Visceral surface woven and lamellar new bone formation on rib fragments of St Benet Sherehog 88.



Figures S3-42. Visceral surface woven and lamellar new bone formation on ribs of Manchester Hanging Ditch 93.



Figure S3-43. Visceral surface woven new bone formation on a rib fragment of Whitefriars 657.



Figure S3-44. Visceral surface woven new bone formation on a rib fragment of Whitefriars 10466.



Figure S3-45. Visceral surface woven new bone formation on ribs of St Peter's Collegiate Church, Wolverhampton 28.



Figure S3-46. Visceral surface woven to lamellar new bone formation on rib fragments of St Peter's Collegiate Church, Wolverhampton 62.

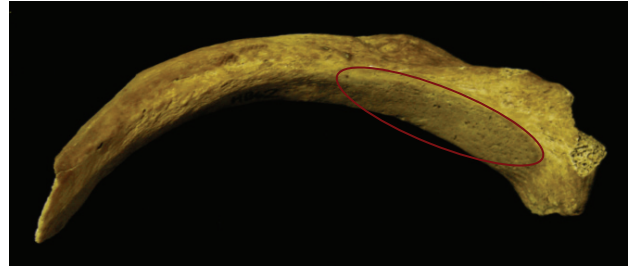


Figure S3-47. Close-up of visceral surface woven to lamellar new bone formation on rib of St Peter's Collegiate Church, Wolverhampton 62.



Figures S3-48. Woven new bone formation on right humerus of St George's Crypt, Leeds 4005.



Figure S3-49. Visceral surface woven and lamellar new bone formation on rib of St George's Crypt, Leeds 4005.

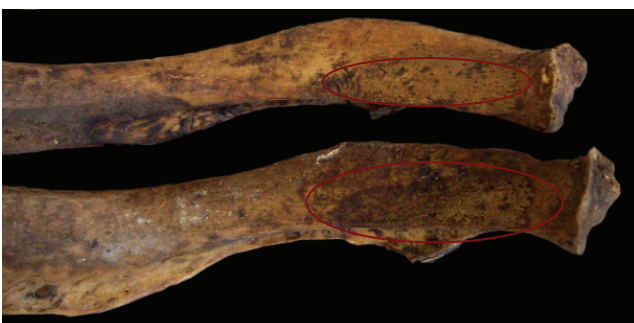


Figure S3-50. Visceral surface woven and lamellar new bone formation on ribs of St George's Crypt, Leeds 4006.

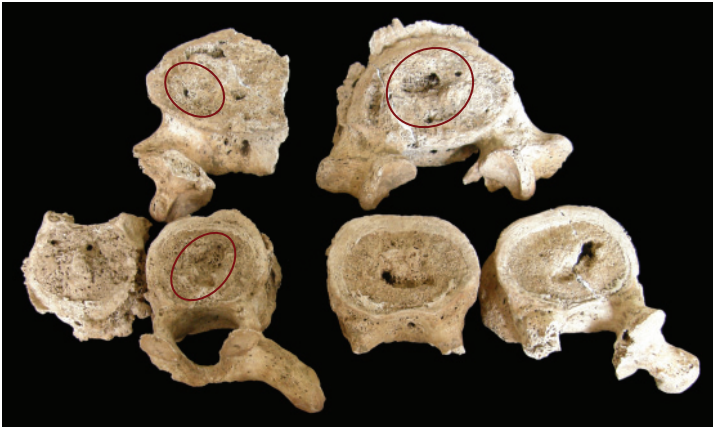


Figure S3-51. Destructive lesions on thoracic and lumbar vertebrae of Slava Rusa 102 .

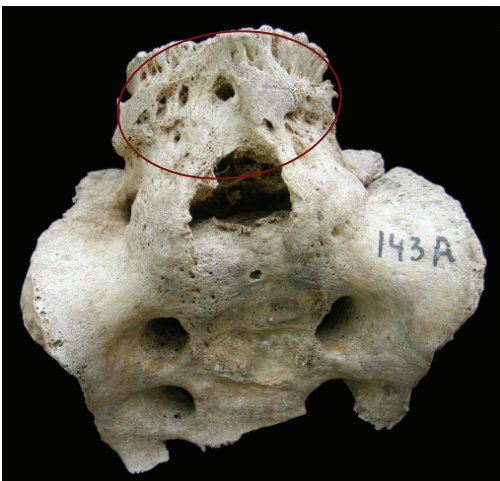


Figure S3-52. Possible TB affecting L4 and L5 of Obelai 143A; destructive lesion.



Figure S3-53. Large area of the cranial bone surface of Shchekavitsa 8 with patches of new bone formation.



Figure S3-54. Visceral surface woven new bone formation on rib fragments of Saint Amé, Douai 20.



Figure S3-55. Destruction of S1, S2 and right sacro-iliac joint in Göttingen 13.k.36.1, and new bone formation on the right ilium.



Figure S3-56. Close-up of Figure S3-55.



Figure S3-57. Destructive lesions in L4 and L5 vertebral bodies of Göttingen 13.k.36.4.